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(54) Title: COMPLETE BIOSYNTHETIC GENE SET FOR SYNTHESIS OF POLYKETIDE ANTIBIOTICS, INCLUDING THE ALBICIDIN FAMILY, RESISTANCE GENES AND USES THEREOF

(57) Abstract: Three gene clusters that together encode albicidin biosynthesis, the complete gene DNA sequences, and the deduced protein sequences for the enzymes and methods for using the DNA sequences are disclosed and discussed as well as methods for plant protection and creating new antibiotics. The novel Albicidin family of antibiotics is disclosed and their structure deduced.



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COMPLETE BIOSYNTHETIC GENE SET FOR SYNTHESIS OF POLYKETIDE
ANTIBIOTICS, INCLUDING THE ALBICIDIN FAMILY,
RESISTANCE GENES, AND USES THEREOF

This application claims the benefit of U.S. Provisional patent application with Serial No. 60/419,463, filed October 18, 2002 the disclosure of which is hereby incorporated by reference in its entirety, including all nucleic acid sequences, amino acid sequences, chemical formulae, tables and figures.

TECHNICAL FIELD

[0001] The invention is in the field of genetic engineering, and in particular the isolation and expression of the biosynthetic genes that produce a family of antibiotics known generically as albicidins.

BACKGROUND OF THE INVENTION

[0002] U.S. Patent No. 4,525,354 to Birch and Patil described a "non-peptide" antibiotic of M.W. "about 842" called "albicidin." Albicidin is described as produced by culturing chlorosis-inducing strains of *Xanthomonas albilineans* isolated from diseased sugarcane, and mutants thereof. The antibiotic was isolated from the culture medium by adsorption on resin and was purified by gel filtration and High Performance Liquid Chromatography (HPLC). The chemical structure of this antibiotic was not determined and remained unknown, although the Birch and Patil patent disclosed spectral data for a fraction having antibiotic activity and the presence of approximately 38 carbon atoms and at least one COOH group.

[0003] *Xanthomonas albilineans* is a systemic, xylem-invading pathogen that causes leaf scald disease of sugarcane (interspecific hybrids of *Saccharum* species) (Ricaud and Ryan, 1989; Rott and Davis, 2000). Leaf scald symptoms include chlorosis, necrosis, rapid wilting, and plant death. Chlorosis-inducing strains of the pathogen produce several toxic compounds. The major toxic component, named albicidin, inhibits chloroplast DNA replication, resulting in blocked chloroplast differentiation and chlorotic leaf streaks that are characteristic of the plant disease (Birch and Patil, 1983, 1985b, 1987a and 1987b). Several studies established that albicidin plays a key role in pathogenesis and especially in the development of disease symptoms (Wall and Birch, 1997; Zhang and Birch, 1997; Zhang *et al.*, 1999; Birch, 2001).

[0004] The prior art indicates that albicidin inhibits prokaryotic DNA replication and is bactericidal to a range of gram-positive and gram-negative bacteria (Birch and Patil, 1985a). Albicidin is therefore of interest as a potential clinical antibiotic (Birch and Patil, 1985a). However, low yield of toxin production in *X. albilineans* has slowed down studies into the chemical structure of albicidin and its therapeutic application (Zhang *et al.*, 1998). The chemical structure of this albicidin remains unknown, however this albicidin has been partially

characterized as a non-peptide antibiotic with a molecular weight of about 842 that contains approximately 38 carbon atoms with three or four aromatic rings, at least one COOH group, two OCH₃ groups, a trisubstituted double bond and a CN linkage (Birch and Patil, 1985a; Huang *et al.*, 2001).

[0005] Molecular cloning and characterization of the genes governing the biosynthesis of albicidin is of considerable interest because such information provides approaches to engineer overproduction of albicidin, to characterize its chemical structure, to allow therapeutic applications and to clarify the relationship between toxin production and the ability to colonize sugarcane. Two similar mutagenesis and complementation studies have been conducted to identify the genetic basis of albicidin production in *X. albilineans* strains isolated in two different geographical locations, Australia and Florida.

[0006] One study of *X. albilineans* strain LS155 from Australia revealed that genes for albicidin biosynthesis and resistance span at least 69kb (Wall and Birch, 1997). Subsequently, three genes required for albicidin biosynthesis were identified, cloned and sequenced from two Australian strains of *X. albilineans* (LS155 and Xa13): *xabA*, *xabB* and *xabC* (Huang *et al.*, 2001; Huang *et al.* 2000a, 2000b). The *xabB* gene encodes a large protein with a predicted size of 525.6 kDa, with a modular architecture indicative of a multi functional polyketide synthase (PKS) linked to a nonribosomal peptide synthetase (NRPS) (Huang *et al.*, 2001). The *xabC* gene, located immediately downstream from *xabB*, encodes an S-adenosyl-L-methionine (SAM)-dependent O-methyltransferase (Huang *et al.*, 2000a). The *xabA* gene, located in another region of the genome, encodes a phosphopantetheinyl transferase required for post-translational activation of PKS and NRPS enzymes (Huang *et al.*, 2000b).

[0007] These first results demonstrated that the albicidin biosynthesis apparatus is a PKS and/or NRPS system. Such systems assemble simple acyl-coenzyme A or amino acid monomers to produce polyketides and/or nonribosomal peptides (Marahiel *et al.*, 1997; Cane, 1997; Cane and Walsh, 1999). These metabolites form very large classes of natural products that include numerous important pharmaceuticals, agrochemicals, and veterinary agents such as antibiotics, immunosuppressants, anti-cholesterolemics, as well as antitumor, antifungal and antiparasitic agents. Genetic studies of prokaryotic PKS and NRPS produced detailed information regarding the function and the organization of genes responsible for the biosynthesis of polyketides and nonribosomal peptides. Such knowledge, in turn, made it possible to produce combinations of PKS and NRPS genes from different microorganisms in order to produce novel antibiotics (McDaniel *et al.*, 1999; Rodriguez and McDaniel, 2001; Pfeifer *et al.*, 2001). Investigating the complete albicidin biosynthesis apparatus is therefore of great interest because such results may contribute to the knowledge as to how PKS and NRPS interact and how they might be manipulated to engineer novel molecules.

[0008] A second study with *X. albilineans* strain Xa23R1 from Florida revealed that at least two gene clusters, one spanning more than 48 kb, are involved in albicidin production (Rott *et al.*, 1996). This conclusion was based on the following data: (i) fifty Xa23R1 mutants defective in albicidin production were isolated; (ii) a Xa23R1 genomic library of 845 clones, designated pALB1 to pALB845, was constructed; (iii) two overlapping DNA inserts of approximately 47 kb and 41 kb, from clones pALB540 and pALB571 respectively, complemented forty-five mutants and were supposed to contain a major gene cluster involved in albicidin production; (iv) a DNA insert of approximately 36 kb, from clone pALB639, complemented four of the five remaining mutants not complemented by pALB540 and pALB571, and was supposed to contain a second region involved in albicidin production; and (v) the remaining mutant, AM37, which was not complemented by any of the three cosmids pALB540, pALB571 and pALB639, was supposed to be mutated in a third region of the genome involved in albicidin production.

[0009] The DNA sequences of all of the genes required to produce the albicidin family of polyketide antibiotics, the expressed protein amino acid sequences of all of the genes, and the deduced structure of Albicidin have not been previously reported, although fragmentary sequences that include three of the biosynthetic genes have been reported. Identification of one albicidin gene, *xabC*, as a methyltransferase gene involved in albicidin biosynthesis is reported by Huang, G., Zhang, L. & Birch, R.G. (2000a, *Gene* **255**, 327-333) and claimed as biologically active in producing a polyketide antibiotic in PCT WO 02/24736 A1. Identification of a second albicidin gene, *xabA*, as a phosphopantetheinyl transferase gene is reported by Huang, G., Zhang, L. and Birch, R.G. (2000b) *Gene* **258**, 193-199 and claimed as biologically active in producing a polyketide antibiotic in PCT WO 02/24736 A1. Huang, G., Zhang, L. & Birch, R.G. (2001) *Microbiology* **147**, 631-642, report a DNA sequence of *xabB* (GenBank accession # AF239749), a multi functional polyketide-peptide synthetase that may be essential for albicidin biosynthesis in *Xanthomonas albilineans*. This *xabB* gene is reported as full length by Birch in PCT WO 02/24736 A1 (their seq. ID #1) and claimed by Birch in PCT WO 02/24736 A1 as a biologically active polyketide synthase of 4,801 amino acids in length, enabling production of albicidin. However, the DNA sequence reported by Huang *et al.* (2001) in GenBank AF239749 and by Birch in PCT WO 02/24736 A1 (their seq. ID #1) appears to be incomplete and missing 6,234 bp of DNA sequence encoding 2,078 amino acids. The subject invention provides the complete DNA sequence of *xabB* (*albI*, our seq. 20) as 20,637 bp, encoding a biologically active polyketide synthase of 6,879 amino acids of in this application (our seq ID #26). Factors affecting biosynthesis by *Xanthomonas albilineans* of albicidins antibiotics and phytotoxins are discussed in *J. Appl. Microbiol.* **85**, 1023-1028. and Wall, M.K. & Birch, R.G. (1997). Genes for albicidin biosynthesis and resistance span at least 69 kb in the genome of *Xanthomonas albilineans*. *Lett. Appl. Microbiol.* **24**, 256-260. A gene from *X. albilineans* strain Xa13, designed AlbF, which confers high level albicidin resistance in *Escherichia coli* and which encodes a putative albicidin

efflux pump, was directly submitted to Genbank by Bostock and Birch (Accession No. AF403709).

SUMMARY OF THE INVENTION

[0010] The present invention describes and characterizes the family of antibiotics that is produced by culturing chlorosis-inducing strains of *X. albilineans* and mutants thereof, together with the complete set of twenty biosynthetic genes capable of producing the unique and previously uncharacterized family of antibiotics produced by *X. albilineans* and previously lumped together as "albicidins." The set of twenty biosynthetic genes isolated, purified and cloned from a culture of *X. albilineans* revealed that this set of biosynthetic genes is capable of synthesizing products exhibiting a high level of variation among the products, indicating that albicidins comprise a family of polyketide antibiotics. The albicidins described in the present invention are synthesized by twenty genes, including one polyketide-peptide synthase, one polyketide synthase and two peptide synthases, but the substrates of the polyketide-peptide synthase and of one peptide synthase are not α -amino acids. The biosynthetic enzymes represent a previously undescribed and unique polyketide antibiotic biosynthetic system.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Figure 1 is a Physical Map and genetic organization of the DNA Region containing the major gene cluster XALB1 involved in the biosynthesis of Albicidins.

[0012] Figure 2 is an illustration of the organization of the four PKS modules and the seven NRPS modules identified in cluster XALB1 and comparison with the organization of the prior art material XabB.

[0013] Figure 3 shows the conserved sequence motifs in O-methyltransferases and C-methyltransferases involved in antibiotic biosynthesis in bacteria and in AlbII.

[0014] Figure 4 shows the conserved sequence motifs in O-methyltransferases and in different tcmP-like hypothetical proteins and AlbVI.

[0015] Figure 5 is an illustration of the alignment of the primary sequences between the conserved motifs A4 and A5 of Alb NPRSs and PKS-4 in *Xanthomonas albilineans* with the corresponding sequences of GrsA (Phe) accession number: P14687 and Blm NRPS-2 (β -Ala) accession number AF210249.

[0016] Figure 6 shows Rho-independent transcription terminators identified in the intergenic regions of XALB1 and XALB3 clusters.

[0017] Figure 7A shows sequences identified as a putative bidirectional promoter between albX and albXVII in XALB1 for transcriptional control of operons 3 and 4.

[0018] Figure 7B shows sequences identified as a putative unidirectional promoter upstream from *albXIX* for transcriptional control of operon 5 if *albXVIII* is not expressed.

[0019] Figure 8 is a physical map and genetic organization of the DNA region containing the gene clusters XALB2 and XALB3 involved in albicidin production.

[0020] Figure 9A is linear model 1 leading to the biosynthesis of only one polyketide-polypeptide albicidin backbone.

[0021] Figure 9B is linear model 2 leading to the biosynthesis of four different polyketide-polypeptide backbone.

[0022] Figure 10A is an alignment of the conserved motifs in AT domains from RifA-1, -2, -3, RifB-1, RifE-1 (Rifamycin PKSs, August *et al.*, 1998) and BlmVIII (Bleomycin PKS; Du *et al.*, 2000).

[0023] Figure 10B is a comparison of AlbXIII, FenF (a malonyl-CoA transacylase located upstream from *mycA*, Duitman *et al.*, 1999) and LipA (a lipase; Valdez *et al.*, 1999).

[0024] Figure 11A is a proposed model for biosynthesis of albicidin, including putative substrates of PKS and NRPS modules.

[0025] Figure 11B shows the proposed compositions and structures of albicidins.

[0026] Figure 12 illustrates subcloning of operons 3 and 4 (from pALB540), XALB2 (from pAC389.1) and XALB3 (from pEV639) into a single plasmid, pOp3-4/XALB2-3. A *Bam*HI-*Pst*I fragment from pALB540, corresponding to a portion of operon 4, was subcloned into pBCKS(+), yielding pBC/Op4D (step 1). A *Xho*I site was introduced into this vector immediately upstream from the *Bfr*I site by directed mutagenesis, yielding pBC/Op4DXhoI (step 2). The *Eco*RI fragment from pAC389.1 (XALB2) was then subcloned into pBC/Op4DXhoI, yielding pBC/Op4D/XALB2 (step 3). A *Bfr*I fragment from pALB540 containing complete operon 3 and the beginning of operon 4 was subcloned into pBC/Op4D/XALB2, yielding pBC/Op3-4/XALB2 (step 4). The *Sal*I fragment from pEV639 (XALB3) was subcloned into pBKS, yielding pBKS/XALB3 (step 5). The *Sal*I site located on the *Kpn*I side of the polylinker was then destroyed and substituted by a *Xho*I restriction site, yielding pBKS/XALB3XhoI (step 6). Finally, the *Xho*I cassette of pBC/Op3-4/XALB2 was subcloned into the *Sal*I restriction site of pBKS/XALB3XhoI, yielding pBKS/Op3-4/XALB2-3 (step 7). An *Xho*I site was added to the *Bam*HI site of pLAFR3, yielding pLAFR3XhoI (step 8). The *Xho*I cassette from pBKS/Op3-4/XALB2-3 was then cloned into pLAFR3XhoI, yielding pOp3-4/XALB2-3 (step 9).

DETAILED DESCRIPTION OF THE INVENTION

[0027] The invention results from the DNA sequencing of the complete major gene cluster XALB1, as well as the noncontiguous fragments XALB2 and XALB3. XALB1 is present in the two overlapping DNA inserts of clones pALB540 and pALB571. Reading frame analysis

and homology analyses allow one to predict the genetic organization of XALB1 and to assign a function to the genes potentially required for albicidin production. Based on the alignment of the different PKS and/or NRPS enzymes encoded by XALB1 we proposed a model for the albicidin backbone biosynthesis. However the invention disclosed herein does not depend upon the accuracy of the proposed model. The invention includes the successful cloning and DNA sequencing of the second region of the genome (XALB2) involved in albicidin production and mutated in mutant AM37.

[0028] The invention includes the characterization of the third region of the genome (XALB3) involved in albicidin production present in clone pALB639. These results allowed the possibility to characterize all enzymes of the albicidin biosynthesis pathway including structural, resistance and regulatory elements and to engineer overproduction of albicidin.

[0029] The subject invention provides:

(a) isolated, recombinant, and/or purified polynucleotide sequences comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25;

(b) isolated, recombinant, and/or purified polynucleotide sequences comprising a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, and 47;

(c) isolated, recombinant, and/or purified polynucleotide sequences comprising a polynucleotide that is complementary to a polynucleotide selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25;

(d) isolated, recombinant, and/or purified polynucleotide sequences comprising a polynucleotide that is complementary to a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, and 47; or

(e) isolated, recombinant, and/or purified polynucleotide sequences comprising a polynucleotide that is at least 70% homologous to: (1) a polynucleotide selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25; (2) a polynucleotide sequence encoding a polypeptide selected from the group consisting of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, and 47; (3) a polynucleotide that is complementary to a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, and 47; (3) a polynucleotide that is complementary to a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25;

(f) isolated, recombinant, and/or purified polynucleotide sequences comprising a polynucleotide sequence encoding variant (e.g., a variant polypeptide) of a polypeptide selected

from the group consisting of SEQ ID NOs: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, and 47, wherein said variant has at least one of the biological activities associated with the polypeptides of SEQ ID NOs: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, and 47;

g) isolated, recombinant, and/or purified polynucleotide sequences comprising polynucleotide sequence encoding a fragment of a polypeptide selected from the group consisting of SEQ ID NOs: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, and 47 or a fragment of a variant polypeptide of SEQ ID NOs: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, and 47;

h) isolated, recombinant, and/or purified polynucleotide sequences comprising a polynucleotide sequence encoding multimeric construct;

j) a genetic construct comprising a polynucleotide sequence as set forth in (a), (b), (c), (d), (e), (f), (g), or (h);

k) a vector comprising a polynucleotide sequence as set forth in (a), (b), (c), (d), (e), (f), (g), or (h);

l) a host cell comprising a vector a polynucleotide sequence as set forth in (a), (b), (c), (d), (e), (f), (g), or (h);

m) a transformed plant cell comprising a vector comprising a polynucleotide sequence as set forth in (a), (b), (c), (d), (e), (f), (g), or (h);

n) a transformed plant comprising a vector comprising a polynucleotide sequence as set forth in (a), (b), (c), (d), (e), (f), (g), or (h); or;

o) a polynucleotide that hybridizes under low, intermediate or high stringency with a polynucleotide sequence as set forth in (a), (b), (c), (d), (e), (f), (g), or (h).

[0030] “Nucleotide sequence”, “polynucleotide” or “nucleic acid” can be used interchangeably and are understood to mean, according to the present invention, either a double-stranded DNA, a single-stranded DNA or products of transcription of the said DNAs (e.g., RNA molecules). It should also be understood that the present invention does not relate to genomic polynucleotide sequences in their natural environment or natural state. The nucleic acid, polynucleotide, or nucleotide sequences of the invention can be isolated, purified (or partially purified), by separation methods including, but not limited to, ion-exchange chromatography, molecular size exclusion chromatography, or by genetic engineering methods such as amplification, subtractive hybridization, cloning, subcloning or chemical synthesis, or combinations of these genetic engineering methods.

[0031] A homologous polynucleotide or polypeptide sequence, for the purposes of the present invention, encompasses a sequence having a percentage identity with the polynucleotide or polypeptide sequences, set forth herein, of between at least (or at least about) 70.00% to 99.99% (inclusive). The aforementioned range of percent identity is to be taken as including, and

providing written description and support for, any fractional percentage, in intervals of 0.01%, between 20.00% and, up to, including 99.99%. These percentages are purely statistical and differences between two nucleic acid sequences can be distributed randomly and over the entire sequence length. For example, homologous sequences can exhibit a percent identity of 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent with the sequences of the instant invention. Typically, the percent identity is calculated with reference to the polynucleotide of a particular SEQ ID NO.; the full-length of a selected polynucleotide, or the native (naturally occurring) polynucleotide. The terms "identical" or percent "identity", in the context of two or more polynucleotide or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithm or by manual alignment and visual inspection.

[0032] A "complementary" polynucleotide sequence, as used herein, generally refers to a sequence arising from the hydrogen bonding between a particular purine and a particular pyrimidine in double-stranded nucleic acid molecules (DNA-DNA, DNA-RNA, or RNA-RNA). The major specific pairings are guanine with cytosine and adenine with thymine or uracil. A "complementary" polynucleotide sequence may also be referred to as an "antisense" polynucleotide sequence or an "antisense" sequence.

[0033] Sequence homology and sequence identity can also be determined by hybridization studies under high stringency, intermediate stringency, and/or low stringency. Various degrees of stringency of hybridization can be employed. The more severe the conditions, the greater the complementarity that is required for duplex formation. Severity of conditions can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under low, intermediate, or high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak [1987] DNA Probes, Stockton Press, New York, NY, pp. 169-170.

[0034] It is also well known in the art that restriction enzymes can be used to obtain functional fragments of the subject DNA sequences. For example, Bal31 exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to as "erase-a-base" procedures). See, for example, Maniatis et al. [1982] Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York; Wei et al. [1983] J. Biol. Chem. 258:13006-13512.

[0035] The present invention further comprises fragments of the polynucleotide sequences of the instant invention. Representative fragments of the polynucleotide sequences according to the invention will be understood to mean any nucleotide fragment having at least 5

successive nucleotides, preferably at least 12 successive nucleotides, and still more preferably at least 15 or at least 20 successive nucleotides of the sequence from which it is derived. The upper limit for such fragments is the total number of nucleotides found in the full-length sequence encoding a particular polypeptide (e.g., a polypeptide selected from the group consisting of SEQ ID NOs: 26-50). The term "successive" can be interchanged with the term "consecutive". In some embodiments, a polynucleotide fragment may be referred to as "a contiguous span of at least X nucleotides, wherein X is any integer value beginning with 5. The upper limit for polynucleotide fragments of the subject invention is the total number of nucleotides found in the full-length sequence of a particular SEQ ID or the total number of nucleotides encoding a particular polypeptide (e.g., a particular SEQ ID NO).

[0036] In some embodiments, the subject invention includes those fragments capable of hybridizing under various conditions of stringency conditions (e.g., high or intermediate or low stringency) with a nucleotide sequence according to the invention; fragments that hybridize with a nucleotide sequence of the subject invention can be, optionally, labeled as set forth below.

[0037] Thus, the subject invention also provides detection probes (e.g., fragments of the disclosed polynucleotide sequences) for hybridization with a target sequence or the amplicon generated from the target sequence. Such a detection probe will comprise a contiguous/consecutive span of at least 8, 9, 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 nucleotides. Labeled probes or primers are labeled with a radioactive compound or with another type of label as set forth above. Alternatively, non-labeled nucleotide sequences may be used directly as probes or primers; however, the sequences are generally labeled with a radioactive element (^{32}P , ^{35}S , ^3H , ^{125}I) or with a molecule such as biotin, acetylaminofluorene, digoxigenin, 5-bromo-deoxyuridine, or fluorescein to provide probes that can be used in numerous applications.

[0038] The subject invention also provides for modified nucleotide sequences. Modified nucleic acid sequences will be understood to mean any nucleotide sequence that has been modified, according to techniques well known to persons skilled in the art, and exhibiting modifications in relation to the native, naturally occurring nucleotide sequences.

[0039] The subject invention also provides genetic constructs comprising: a) a polynucleotide sequence encoding a polypeptide sequence selected from the group consisting of SEQ ID NOs: 1-25; b) a polynucleotide sequence having at least about 70% to 99.99% identity to a polynucleotide sequence encoding a polypeptide sequence selected from the group consisting of SEQ ID NO: 26-50, wherein said polynucleotide encodes a polypeptide having at least one of the biological activities of the polypeptides (e.g., a catalytic activity as set forth in Table 4); c) a polynucleotide sequence encoding a biologically active fragment of a polypeptide selected from the group consisting of SEQ ID NO: 26-50, wherein said biologically active fragment has at least

one of the biological activities of the polypeptides (e.g., a catalytic or transport activity as set forth in Table 4); d) a polynucleotide sequence comprising SEQ ID NO: 1, 2, 3, or combinations thereof; e) a polynucleotide sequence encoding variant (e.g., a variant polypeptide) of a polypeptide selected from the group consisting of SEQ ID NOs: 26-48, wherein said variant has at least one of the biological activities associated with the polypeptides (e.g., a catalytic or transport activity as set forth in Table 4); f) a polynucleotide sequence encoding a fragment of a variant polypeptide as set forth in (e); or g) a polynucleotide sequence encoding multimeric construct. Genetic constructs of the subject invention can also contain additional regulatory elements such as promoters and enhancers and, optionally, selectable markers.

[0040] Also within the scope of the subject instant invention are vectors or expression cassettes containing polynucleotides encoding the polypeptides, set forth supra, operably linked to regulatory elements. The vectors and expression cassettes may contain additional transcriptional control sequences as well. The vectors and expression cassettes may further comprise selectable markers. The expression cassette may contain at least one additional gene, operably linked to control elements, to be co-transformed into the organism. Alternatively, the additional gene(s) and control element(s) can be provided on multiple expression cassettes. Such expression cassettes are provided with a plurality of restriction sites for insertion of the sequences of the invention to be under the transcriptional regulation of the regulatory regions. The expression cassette(s) may additionally contain selectable marker genes operably linked to control elements.

[0041] In some embodiments, the expression cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region, a DNA sequence of the invention, and a transcriptional and translational termination region functional in plants. The transcriptional initiation region, the promoter, may be native or analogous, or foreign or heterologous, to the plant host. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. By "foreign" is intended that the transcriptional initiation region is not found in the native plant into which the transcriptional initiation region is introduced. As used herein, a chimeric gene comprises a coding sequence operably linked to a transcriptional initiation region that is heterologous to the coding sequence.

[0042] The termination region may be native with the transcriptional initiation region, may be native with the operably linked DNA sequence of interest, or may be derived from another source. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also Guerineau et al. (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot (1991) *Cell* 64:671-674; Sanfacon et al. (1991) *Genes Dev.* 5:141-149; Mogen et al. (1990) *Plant Cell* 2:1261-1272; Munroe et al. (1990) *Gene* 91:151-158; Ballas et al. (1989) *Nucleic Acids Res.* 17:7891-7903; and Joshi et al. (1987) *Nucleic Acid Res.* 15:9627-9639.

[0043] Where appropriate, the polynucleotides encoding the polypeptides set forth supra can be optimized for expression in the transformed plant. That is, the genes can be synthesized using plant-preferred codons corresponding to the plant of interest. Methods are available in the art for synthesizing plant-preferred genes. See, for example, U. S. Patent Nos. 5,380,831 and 5,436,391, and Murray et al. (1989) *Nucleic Acids Res.* 17:477-498, herein incorporated by reference.

[0044] The expression cassettes may additionally contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region), Elroy-Stein et al. (1989) *PNAS USA* 86:6126-6130; potyvirus leaders, for example, TEV leader (Tobacco Etch Virus), Allison et al. (1986); MDMV Leader (Maize Dwarf Mosaic Virus), *Virology* 154:9-20; human immunoglobulin heavy-chain binding protein (BiP), Macejak et al. (1991) *Nature* 353:90-94; untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4), Jobling et al. (1987) *Nature* 325:622-625; tobacco mosaic virus leader (TMV), Gallie et al. (1989) in *Molecular Biology of RNA*, ed. Cech (Liss, New York), pp. 237-256; and maize chlorotic mottle virus leader (MCMV), Lommel et al. (1991) *Virology* 81:382-385. See also, Della-Cioppa et al. (1987) *Plant Physiol.* 84:965-968. Other methods known to enhance translation can also be utilized.

[0045] Also provided are transformed host cells, transformed plant cells and transgenic plants which contain one or more genetic constructs, vectors, or expression cassettes comprising polynucleotides of the subject invention, or biologically active fragments thereof, operably linked to control elements. As used herein, the term "planta" includes algae and higher plants. Thus, algae, monocots, and dicots may be transformed with genetic constructs of the invention, expression cassettes, or vectors according to the invention. In certain embodiments of the subject invention, the transformed cells or transgenic plants comprise at least one polynucleotide sequence selected from the group consisting of SEQ ID NOs: 1-25. In certain preferred embodiments, transformed cells or transgenic plants comprise at least one polynucleotide sequence comprising SEQ ID NO: 1, 2, or 3. Optionally, the transformed cells or transgenic plants can comprise at least two or all three polynucleotide sequences selected from the group consisting of SEQ ID NOs: 1, 2, and 3.

[0046] Methods of transforming cells with genetic constructs, vectors, or expression cassettes comprising the novel polynucleotides of the invention are also provided. These methods comprise transforming a plant or plant cell with a polynucleotide according to the subject invention. Plants and plant cells may be transformed by electroporation, *Agrobacterium* transformation (including vacuum infiltration), engineered plant virus replicons, electrophoresis, microinjection, micro-projectile bombardment, vacuum infiltration of *Agrobacterium*, micro-

LASER beam-induced perforation of cell wall, or simply by incubation with or without polyethylene glycol (PEG). Plants transformed with a genetic construct of the invention may be produced by standard techniques known in the art for the genetic manipulation of plants. DNA can be transformed into plant cells using any suitable technology, such as a disarmed Ti-plasmid vector carried by *Agrobacterium* exploiting its natural gene transferability. *Agrobacterium* transformation is used by those skilled in the art to transform algae and dicotyledonous species. Substantial progress has been made towards the routine production of stable, fertile transgenic plants in almost all economically relevant monocot plants. In particular, *Agrobacterium* mediated transformation has now emerged as a highly efficient transformation method in monocots. Microprojectile bombardment, electroporation, and direct DNA uptake are preferred where *Agrobacterium* is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, e.g., bombardment with *Agrobacterium*-coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with *Agrobacterium* (EP-A-486233).

[0047] Following transformation, a plant may be regenerated, e.g., from single cells, callus tissue or leaf discs, as is standard in the art. Almost any plant can be entirely regenerated from cells, tissues, and organs of the plant. Available techniques are reviewed in Vasil et al. (1984) in *Cell Culture and Somatic Cell Genetics of Plants*, Vols. I, II, and III, Laboratory Procedures and Their Applications (Academic press); and Weissbach et al. (1989) *Methods for Plant Mol. Biol.*

[0048] The transformed plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and inherited, and then seeds harvested to ensure expression of the desired phenotypic characteristic has been achieved.

[0049] The particular choice of a transformation technology will be determined by its efficiency to transform certain plant species as well as the experience and preference of the person practicing the invention with a particular methodology of choice. It will be apparent to the skilled person that the particular choice of a transformation system to introduce nucleic acid into plant cells is not essential to or a limitation of the invention, nor is the choice of technique for plant regeneration.

[0050] Also according to the invention, there is provided a plant cell having the constructs of the invention. A further aspect of the present invention provides a method of making such a plant cell involving introduction of a vector including the construct into a plant cell. For integration of the construct into the plant genome, such introduction will be followed by recombination between the vector and the plant cell genome to introduce the sequence of

nucleotides into the genome. RNA encoded by the introduced nucleic acid construct may then be transcribed in the cell and descendants thereof, including cells in plants regenerated from transformed material. A gene stably incorporated into the genome of a plant is passed from generation to generation to descendants of the plant, so such descendants should show the desired phenotype.

[0051] The present invention also provides a plant comprising a plant cell as disclosed. Transformed seeds and plant parts are also encompassed. As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to naturally occurring, deliberate, or inadvertent caused mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

[0052] In addition to a plant, the present invention provides any clone of such a plant, seed, or hybrid descendants, and any part of any of these, such as cuttings or seed. The invention provides any plant propagule that is any part which may be used in reproduction or propagation, sexual or asexual, including cuttings, seed, and so on. Also encompassed by the invention is a plant which is a sexually or asexually propagated off-spring, clone, or descendant of such a plant; or any part or propagule of said plant, off-spring, clone, or descendant. Plant extracts and derivatives are also provided.

[0053] As is apparent to the routineer in this technology, the disclosed methods allow for the expression of a gene of interest in any plant. The invention thus relates generally to methods for the production of transgenic plants (both monocots and dicots). As used herein, the term "transgenic plants" refers to plants (algae, monocots, or dicots), comprising plant cells in which homologous or heterologous polynucleotides are expressed as the result of manipulation by the hand of man.

[0054] As is apparent to one of ordinary skill in the art, the peptides encoded by the disclosed herein may be encoded by multiple polynucleotide sequences because of the redundancy of the genetic code. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding the same, or essentially the same, amino acid sequences. These variant DNA sequences are within the scope of the subject invention.

[0055] The terms "purified" and "isolated", when referring to a polynucleotide, nucleotide, or nucleic acid, indicate a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term therefore covers, for example, (a)

a DNA which has the sequence of part of a naturally occurring genomic DNA molecules but is not flanked by both of the coding or non-coding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs (e.g., DNA excised with a restriction enzyme); (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids present in mixtures of (i) DNA molecules, (ii) transfected cells, and (iii) cell clones, e.g., as these occur in a DNA library such as a cDNA or genomic DNA library.

[0056] The term "polynucleotide" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule and thus includes double- and single-stranded DNA and RNA. It also includes known types of modifications, for example, labels which are known in the art, methylation, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications, such as those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as proteins (including for e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide.

[0057] "Control elements" include both "transcriptional control elements" and "translational control elements". "Transcriptional control elements" include "promoter", "enhancer", and "transcription termination" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription [Maniatis et al. [1987] Science 236:1237]. Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in plants, yeast, insect and mammalian cells and viruses (analogous control elements, i.e., promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the peptide of interest. Some eukaryotic promoters and enhancers have a broad host range while others are functional in a limited subset of cell types [for review see Voss et al. [1986] Trends Biochem. Sci. 11:287 and Maniatis et al. [1987] supra. Transcriptional control elements suitable for use in plants are well known in the art. "Translational control elements" include translational initiation regions and translational termination regions functional in plants.

[0058] A number of promoters can be used in the practice of the invention. The promoters can be selected based on the desired outcome. Strong promoters may be used to produce high levels of gene transcription. Alternatively, inducible promoters may be used to selectively active gene transcription when the appropriate signal is provided. Constitutive promoters may be utilized to continuously drive gene transcription. Tissue specific promoters may also be used in the practice of the invention in order to provide localized production of gene transcripts in a desired tissue. Developmental promoters may, likewise, be used to drive transcription of a gene during a particular developmental stage of the plant. Thus, a gene of interest can be combined with constitutive, tissue-specific, inducible, developmental, or other promoters for expression in plants depending upon the desired outcome.

[0059] Constitutive promoters include, for example, CaMV 35S promoter (Odell et al. (1985) *Nature* 313:810-812; rice actin (McElroy et al. (1990) *Plant Cell* 2:163-171; ubiquitin (Christensen et al. (1989) *Plant Mol. Biol.* 12:619-632 and Christensen et al. (1992) *Plant Mol. Biol.* 18:675-689); pEMU (Last et al. (1991) *Theor. Appl. Genet.* 81:581-588); MAS (Velten et al. (1984) *EMBO J.* 3:2723-2730); ALS promoter (U. S. Patent No. 5,659,026), and the like. Other constitutive promoters include those in U. S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; and 5,608,142. Each of the aforementioned patents and references is hereby incorporated by reference in its entirety.

[0060] A number of inducible promoters are known in the art. For example, a pathogen-inducible promoter can be utilized. Such promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen; e.g., PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc. See, for example, Redolfi et al. (1983) *Neth. J. Plant Pathol.* 89:245-254; Uknes et al. (1992) *Plant Cell* 4:645-656; and Van Loon (1985) *Plant Mol. Virol.* 4:111-116; Marineau et al. (1987) *Plant Mol. Biol.* 9:335-342; Matton et al. (1989) *Molecular Plant-Microbe Interactions* 2:325-331; Somsisch et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:2427-2430; Somsisch et al. (1988) *Mol. Gen. Genet.* 2:93-98; and Yang (1996) *Proc. Natl. Acad. Sci. USA* 93:14972-14977. See also, Chen et al. (1996) *Plant J.* 10:955-966; Zhang et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:2507-2511; Warner et al. (1993) *Plant J.* 3:191-201; Siebertz et al. (1989) *Plant Cell* 1:961-968; U. S. Patent No. 5,750,386; Cordero et al. (1992) *Physiol. Mol. Plant Path.* 41:189-200; each of which is incorporated by reference in its entirety.

[0061] Wound-inducible promoters may be used in the genetic constructs of the invention. Such wound-inducible promoters include potato proteinase inhibitor (pin II) gene (Ryan (1990) *Ann. Rev. Phytopath.* 28:425-449; Duan et al. (1996) *Nature Biotechnology* 14:494-498; wun1 and wun2, U. S. Patent No. 5,428,148; win1 and win2 (Stanford et al. (1989) *Mol. Gen. Genet.* 215:200-208); systemin (McGurl et al. (1992) *Science* 225:1570-1573); WIP1 (Rohmeier et al. (1993) *Plant Mol. Biol.* 22:783-792; Eckelkamp et al. (1993) *FEBS Letters*

323:73-76); MPI gene (Corderok et al. (1994) *Plant J.* 6(2):141-150; and the like. These references are also incorporated by reference in their entireties.

[0062] Tissue specific promoters can also be used in the practice of the subject invention. For example, leaf-specific promoters can similarly be used if desired, and are taught in references which include Yamamoto et al. (1997) *Plant J.* 12(2):255-265; Kawamata et al. (1997) *Plant Cell Physiol.* 38(7):792-803; Hansen et al. (1997) *Mol. Gen. Genet.* 254(3):337-343; Russel et al. (1997) *Transgenic Res.* 6(2):157-168; Rinehart et al. (1996) *Plant Physiol.* 112(3):1331-1341; Van Camp et al. (1996) *Plant Physiol.* 112(2):525-535; Canevascini et al. (1996) *Plant Physiol.* 112(2):513-524; Yamamoto et al. (1994) *Plant Cell Physiol.* 35(5):773-778; Lam (1994) *Results Probl. Cell Differ.* 20:181-196; Orozco et al. (1993) *Plant Mol. Biol.* 23(6):1129-1138; Matsuoka et al. (1993) *Proc. Natl. Acad. Sci USA*:90(20) 9586-9590; and Guevara-Garcia et al. (1993) *Plant J.* 4(3):495-505. Alternatively, root-specific promoters are known and can be selected from the many available from the literature. See, for example, Hire et al. (1992) *Plant Mol. Biol.* 20(2):207-218 (soybean root-specific glutamine synthetase gene); Keller and Baumgartner (1991) *Plant Cell* 3(10):1051-1061 (root-specific control element in the GRP 1.8 gene of French bean); Sanger et al. (1990) *Plant Mol. Biol.* 14(3):433-443 (root-specific promoter of the mannopine synthase (MAS) gene of *Agrobacterium tumefaciens*); Miao et al. (1991) *Plant Cell* 3(1):11-22 (full-length cDNA clone encoding cytosolic glutamine synthetase (GS), which is expressed in roots and root nodules of soybean). Bogusz et al. (1990) *Plant Cell* 2(7):633-641 (root specific promoters from hemoglobin genes from the nitrogen-fixing nonlegume *Parasponia andersonii* and the related non-nitrogen-fixing nonlegume *Trema tomeniosa*); Leach and Aoyagi (1991) *Plant Science (Limerick)* 79(1):69-76 (rolC and rolD root-including genes of *Agrobacterium rhizogenes*); Teeri et al. (1989) *EMBO J.* 8(2):343-350 (octopine synthase and TR2' gene); (VfENOD-GRP3 gene promoter); Kuster et al. (1995) *Plant Mol. Biol.* 29(4):759-772 and Capana et al. (1994) *Plant Mol. Biol.* 25(4):681-691 (rolB promoter). See also U. S. Patent Nos. 5,837,876; 5,750,386; 5,633,363; 5,459,252; 5,401,836; 5,110,732; and 5,023,179.

[0063] Other tissue specific promoters can also be used in the practice of the subject invention (see, for example U.S. Patent No. 6,544,783). For example, xylem/vascular/tracheid-specific promoters, such as those disclosed in Milioni et al. (2002) *Plant Cell*, 14:2813-2824; Zhong et al. (1999) *Plant Cell*, 11:2139-2152; Ito et al. (2002) *Plant Cell*, 14:3201-3211; Parker et al. (2003) *Development* 130:2139-2148; Bourquin et al. (2002) *Plant Cell* 14:3073-3088 (each of which is hereby incorporated by reference in its entirety) can be used in the practice of the subject invention.

[0064] "Seed-preferred" promoters include both "seed-specific" promoters (those promoters active during seed development such as promoters of seed storage proteins) as well as "seed-germinating" promoters (those promoters active during seed germination). See Thompson

et al. (1989) Bioassays 10:108, herein incorporated by reference. Such seed-preferred promoters include, but are not limited to, Cim1 (cytokinin-induced message); cZ10B1 (Maize 19 kDa zein); ceaA (cellulose synthase); gama-zein; Glob-1; bean β -phaseolin; napin; β -conglycinin; soybean lectin; cruciferin; maize 15 kDa zein; 22 kDa zein; 27 kDa zein; g-zein; waxy; shrunken 1; shrunken 2; globulin 1; etc.

[0065] "Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

[0066] As used herein, the term "expression cassette" refers to a molecule comprising at least one coding sequence operably linked to a control sequence which includes all nucleotide sequences required for the transcription of cloned copies of the coding sequence and the translation of the mRNAs in an appropriate host cell. Such expression cassettes can be used to express eukaryotic genes in a variety of hosts such as bacteria, green algae, cyanobacteria, plant cells, fungal cells, yeast cells, insect cells and animal cells. Under the invention, expression cassettes can include, but are not limited to, cloning vectors, specifically designed plasmids, viruses or virus particles. The cassettes may further include an origin of replication for autonomous replication in host cells, selectable markers, various restriction sites, a potential for high copy number and strong promoters.

[0067] By "vector" is meant any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, virus etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

[0068] During the preparation of the constructs, the various fragments of DNA will often be cloned in an appropriate cloning vector, which allows for amplification of the DNA, modification of the DNA or manipulation of the DNA by joining or removing sequences, linkers, or the like. Preferably, the vectors will be capable of replication to at least a relatively high copy number in *E. coli*. A number of vectors are readily available for cloning, including such vectors as pBR322, vectors of the pUC series, the M13 series vectors, and pBluescript vectors (Stratagene; La Jolla, Calif.).

[0069] In order to provide a means of selecting transformed plants or plant cells, the vectors for transformation will typically contain a selectable marker gene. Marker genes are expressible DNA sequences which express a polypeptide which resists a natural inhibition by, attenuates, or inactivates a selective substance. Examples of such substances include antibiotics and, in the case of plant cells, herbicides. Selectable markers for use in animal, bacterial, plant, fungal, yeast, and insect cells are well known in the art. Exemplary selectable markers include

bacterial transposons Tn5 or Tn 601(903) conferring resistance to aminoglycosides (selection for Geneticin-resistance (G418R), mycophenolic acid resistance (MPAR) (utilizing *E. coli* guanosine phosphoribosyl transferase (*gpt*) encoding the enzyme XGPRT; selection is performed on medium containing MPA and xanthin), methotrexate resistance (MTXR), or cadmium-resistance which incorporates the mouse metallothionein gene (as cDNA cassette) on the vector which detoxifies heavy metal ions by chelating them.

[0070] Alternatively, a marker gene may provide some visible indication of cell transformation. For example, it may cause a distinctive appearance or growth pattern relative to plants or plant cells not expressing the selectable marker gene in the presence of some substance, either as applied directly to the plant or plant cells or as present in the plant or plant cell growth media. The use of such a marker for identification of plant cells containing a plastid construct has been described (Svab et al. [1993] *supra*). Numerous additional promoter regions may also be used to drive expression of the selectable marker gene, including various plant promoters and bacterial promoters which have been shown to function in plants.

[0071] A number of other markers have been developed for use with plant cells, such as resistance to chloramphenicol, the aminoglycoside G418, hygromycin, or the like. Other genes which encode a product involved in chloroplast metabolism may also be used as selectable markers. For example, genes which provide resistance to plant herbicides such as glyphosate, bromoxynil or imidazolinone may find particular use. Such genes have been reported (Stalker et al. [1985] *J. Biol. Chem.* 260:4724-4728 (glyphosate resistant EPSP); Stalker et al. [1985] *J. Biol. Chem.* 263:6310-6314 (bromoxynil resistant nitrilase gene); and Sathasivan et al. [1990] *Nucl. Acids Res.* 18:2188 (AHAS imidazolinone resistance gene)).

[0072] Another aspect of the invention provides vectors for the cloning and/or the expression of a polynucleotide sequences taught herein in procaryotic or animal cells. The subject invention also provides for the expression of a polypeptide, peptide, derivative, or variant encoded by a polynucleotide sequence disclosed herein comprising the culture of a procaryotic or animal cell (a host cell) transformed with a polynucleotide of the subject invention under conditions that allow for the expression of a polypeptide, biologically active fragment, or multimeric construct encoded by said polynucleotide and, optionally, recovering the expressed polypeptide, peptide, derivative, or analog.

[0073] In this aspect of the invention, the polynucleotide sequences can be regulated by a second nucleic acid sequence so that the protein or peptide is expressed in a host cell transformed with the recombinant DNA molecule. For example, expression of a protein or peptide may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression include, but are not limited to, the CMV-IE promoter, the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long

terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes simplex thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic vectors containing promoters such as the β -lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella et al., 1983, Nature 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, et al., 1981, Nucl. Acids Res. 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310:115-120); promoter elements from yeast or fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, and/or the alkaline phosphatase promoter.

[0074] The vectors according to the invention are, for example, vectors of plasmid or viral origin. In a specific embodiment, a vector is used that comprises a promoter operably linked to a nucleic acid sequence encoding a polypeptide as disclosed herein, one or more origins of replication, and, optionally, one or more selectable markers (e.g., an antibiotic resistance gene). Expression vectors comprise regulatory sequences that control gene expression, including gene expression in a desired host cell. Exemplary vectors for the expression of the polypeptides of the invention include the pET-type plasmid vectors (Promega) or pBAD plasmid vectors (Invitrogen) or those provided in the examples below. Furthermore, the vectors according to the invention are useful for transforming host cells so as to clone or express the polynucleotide sequences of the invention.

[0075] The invention also encompasses the host cells transformed by a vector according to the invention. These cells may be obtained by introducing into host cells a nucleotide sequence inserted into a vector as defined above, and then culturing the said cells under conditions allowing the replication and/or the expression of the polynucleotide sequences of the subject invention.

[0076] The host cell may be chosen from eukaryotic or prokaryotic systems, such as for example bacterial cells, (Gram negative or Gram positive), yeast cells (for example, *Saccharomyces cerevisiae* or *Pichia pastoris*), animal cells (such as Chinese hamster ovary (CHO) cells), plant cells (e.g., algae), and/or insect cells using baculovirus vectors. In some embodiments, the host cells for expression of the polypeptides include, and are not limited to, those taught in U.S. Patent Nos. 6,319,691, 6,277,375, 5,643,570, or 5,565,335, each of which is incorporated by reference in its entirety, including all references cited within each respective patent.

[0077] Furthermore, a host cell strain may be chosen which modulates the expression of

the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, phosphorylation) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can also to provide glycosylation of a protein.

[0078] The subject invention provides one or more isolated polypeptides comprising:

- (a) SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47;
- (b) a heterologous polypeptide sequence fused, in frame, to a polypeptide comprising SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47;
- (c) a fragment of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47, wherein said fragment exhibits at least one biological function of the polypeptide of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47; or
- (d) a variant having at least 70% homology to a polypeptide comprising SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47, wherein said variant exhibits at least one biological function of the polypeptide comprising SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47.

[0079] The term “peptide” may be used interchangeably with “oligopeptide” or “polypeptide” in the instant specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α -amino and carboxyl groups of adjacent amino acids. Linker elements can be joined to the polypeptides of the subject invention through peptide bonds or via chemical bonds (e.g., heterobifunctional chemical linker elements).

[0080] The subject invention encompasses polypeptide fragments of the full-length polypeptides disclosed herein. Polypeptide fragments, according to the subject invention, usually comprise a contiguous span of at least 5 consecutive (or contiguous) amino acids. The maximum length for a polypeptide fragment in the context of this invention is an integer that is one amino acid less than the full length of a particular SEQ ID NO: from which the fragment was derived. In certain preferred embodiments, fragments of the polypeptides of the subject invention retain at least one biological activity/function of the full-length polypeptide from which they are derived

(e.g., such similar or identical enzymatic activity or the ability to provide resistance to an antibiotic or transport an antibiotic out of a cell (see, for example, Table 4).

[0081] A “variant” polypeptide (or polypeptide variant) is to be understood to designate polypeptides exhibiting, in relation to the natural polypeptide, certain modifications. These modifications can include a deletion, addition, or substitution of at least one amino acid, a truncation, an extension, a chimeric fusion, a mutation, or polypeptides exhibiting post-translational modifications. Among the homologous polypeptides, those whose amino acid sequences exhibit between at least (or at least about) 70.00% to 99.99% (inclusive) identity to the full length, native, or naturally occurring polypeptide are another aspect of the invention. The aforementioned range of percent identity is to be taken as including, and providing written description and support for, any fractional percentage, in intervals of 0.01%, between 70.00% and, up to, including 99.99%. These percentages are purely statistical and differences between two polypeptide sequences can be distributed randomly and over the entire sequence length. Thus, variant polypeptides can have 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity with the polypeptide sequences of the instant invention. In certain preferred embodiments, variants of the polypeptides of the subject invention retain at least one biological activity/function of the full-length polypeptide from which they are derived (e.g., such as similar or identical enzymatic activity or the ability to provide resistance to an antibiotic or transport an antibiotic out of a cell (see, for example, Table 4).

[0082] Variant polypeptides can also comprise one or more heterologous polypeptide sequences (e.g., tags that facilitate purification of the polypeptides of the invention (see, for example, U.S. Patent No. 6,342,362, hereby incorporated by reference in its entirety; Altendorf et al. [1999-WWW, 2000] “Structure and Function of the Fo Complex of the ATP Synthase from Escherichia Coli,” J. of Experimental Biology 203:19-28, The Co. of Biologists, Ltd., G.B.; Baneyx [1999] “Recombinant Protein Expression in Escherichia coli,” Biotechnology 10:411-21, Elsevier Science Ltd.; Eihauer et al. [2001] “The FLAG™ Peptide, a Versatile Fusion Tag for the Purification of Recombinant Proteins,” J. Biochem Biophys Methods 49:455-65; Jones et al. [1995] J. Chromatography 707:3-22; Jones et al. [1995] “Current Trends in Molecular Recognition and Bioseparation,” J. of Chromatography A. 707:3-22, Elsevier Science B.V.; Margolin [2000] “Green Fluorescent Protein as a Reporter for Macromolecular Localization in Bacterial Cells,” Methods 20:62-72, Academic Press; Puig et al. [2001] “The Tandem Affinity Purification (TAP) Method: A General Procedure of Protein Complex Purification,” Methods 24:218-29, Academic Press; Sassenfeld [1990] “Engineering Proteins for Purification,” TibTech 8:88-93; Sheibani [1999] “Prokaryotic Gene Fusion Expression Systems and Their Use in Structural and Functional Studies of Proteins,” Prep. Biochem. & Biotechnol. 29(1):77-90, Marcel Dekker, Inc.; Skerra et al. [1999] “Applications of a Peptide Ligand for Streptavidin: the Strep-

tag", Biomolecular Engineering 16:79-86, Elsevier Science, B.V.; Smith [1998] "Cookbook for Eukaryotic Protein Expression: Yeast, Insect, and Plant Expression Systems," The Scientist 12(22):20; Smyth et al. [2000] "Eukaryotic Expression and Purification of Recombinant Extracellular Matrix Proteins Carrying the Strep II Tag", Methods in Molecular Biology, 139:49-57; Unger [1997] "Show Me the Money: Prokaryotic Expression Vectors and Purification Systems," The Scientist 11(17):20, each of which is hereby incorporated by reference in their entireties), or commercially available tags from vendors such as STRATAGENE (La Jolla, CA), NOVAGEN (Madison, WI), QIAGEN, Inc., (Valencia, CA), or InVitrogen (San Diego, CA). Alternatively, the heterologous sequences may provide for the multimerization of the polypeptides of the subject invention (see, e.g., US Patent Number 5,478,925, WO 98/49305, or U.S. Pat. No. 5,073,627, Landschulz *et al.*, (1988), Science. 240:1759, WO 94/10308, Hoppe *et al.*, (1994), FEBS Letters. 344:191). Other methods of making multimers include the addition of cysteine or biotin to the C-terminus or N-terminus of the polypeptide using techniques known in the art. Where biotin is attached to a polypeptide, avidin can be utilized to create multimers of the polypeptides to which the biotin element is attached (see, e.g., US Patent Number 5,478,925 for numerous methods of multimerization). Multimers of the invention may also be generated using chemical or genetic engineering techniques known in the art.

[0083] The invention, thus, provides a novel antibiotic family, Albicidins, produced by three novel biosynthetic gene clusters (XALB1, XALB2, and XALB3) contained within a host cell DNA in which one strand comprises non-contiguously SEQ. ID No. 1, SEQ. ID No. 2 and SEQ ID No. 3, and the cell expresses the DNA to provide peptides including those named AlbI (SEQ ID No. 26) (encoded by SEQ ID No. 20), AlbII (SEQ ID No. 27) (encoded by SEQ ID No. 21), AlbIII (SEQ ID No. 28) (encoded by SEQ ID No. 22), AlbIV (SEQ ID No. 29) (encoded by SEQ ID No. 23), AlbVI (SEQ ID No. 31) (encoded by SEQ ID No. 18), AlbVII (SEQ ID No. 32) (encoded by SEQ ID No. 17), AlbVIII (SEQ ID No. 33) (encoded by SEQ ID No. 16), AlbIX (SEQ ID No. 34) (encoded by SEQ ID No. 15), AlbX (SEQ ID No. 35) (encoded by SEQ ID No. 10), AlbXI (SEQ ID No. 36) (encoded by SEQ ID No. 9), AlbXII (SEQ ID No. 37) (encoded by SEQ ID No. 8), AlbXIII (SEQ ID No. 38) (encoded by SEQ ID No. 7), AlbXIV (SEQ ID No. 39) (encoded by SEQ ID No. 6), AlbXV (SEQ ID No. 40) (encoded by SEQ ID No. 5), AlbXVII (SEQ ID No. 42) (encoded by SEQ ID No. 11), AlbXVIII (SEQ ID No. 43) (encoded by SEQ ID No. 12), AlbXIX (SEQ ID No. 44) (encoded by SEQ ID No. 13), AlbXX (SEQ ID No. 45) (encoded by SEQ ID No. 14), AlbXXI (SEQ ID No. 46) (encoded by SEQ ID No. 24), and AlbXXII (SEQ ID No. 47) (encoded by SEQ ID No. 25), that in turn interact within the host cell to produce one or more antibiotics as more fully illustrated in Figure 11.

[0084] In one embodiment, the invention comprises a plurality of isolated and purified DNA strands which comprise nucleotide sequences selected from the group consisting of SEQ ID

No: 1 to SEQ. ID No. 25, each individual sequence, except the transposases AlbV (SEQ ID No. 30) (encoded by SEQ ID No. 19) and AlbXVI (SEQ ID No. 41) (encoded by SEQ ID No. 4) found in the XALB1 cluster, being necessary to the biosynthesis of the novel family of antibiotics, Albicidins.

[0085] The invention also includes the peptides or proteins encoded by the genes of the biosynthetic complex expressed by the combination of DNA with a strand having sequences SEQ ID Nos. 1 to 3. Proteins are named with roman numerals and the prefix Alb from AlbI to Alb XXII have the amino acid sequences of SEQ ID Nos. 26 to 47 (not in Roman numeral order but in the order of placement of the genes within sequences SEQ ID Nos. 1 to 3 that express each protein). Expression of the peptides having the amino acid sequences of SEQ ID Nos. 26 to 29, 31 to 40 and 42 to 47, have been found to be all required for the successful biosynthesis of Albicidins.

[0086] The invention further provides a method for producing Albicidins comprising providing a modified host cell with a heterologous DNA Albicidin Biosynthetic Gene Cluster or set of genes defined as DNA operably comprising DNA sequences substantially similar to SEQ ID Nos. 1 to 3. Substantially the same means DNA having sufficient homology to provide expressed proteins that function to provide an antibiotic material having the structural components identified herein. Preferably a given sequence will have at least 70 percent homology to one of SEQ ID Nos. 1 to 3, preferably 85% homology and most preferably at least 95% homology. The method includes the steps of modifying the DNA of the host cell to comprise an operable expression system for maintaining the modified host cell under conditions supporting biosynthesis of Albicidins and isolation of Albicidins from the host cell or its environment. The invention further provides a method of production of a group of novel antibiotic materials utilizing at least three of the Sequences selected from the group consisting of DNA SEQ ID No. 1 to SEQ ID No. 25 (excluding transposases encoded by SEQ ID Nos. 4 and 19) inclusive in combination with additional sequences to produce a modified Albicidin-like material.

[0087] More specifically, the invention provides DNA Sequences comprising at least about 68,498 base pairs and including an about 55,839 bp region from the genome of *X. albilineans* designated as XALB1 (Albicidin Biosynthetic Gene Cluster 1; SEQ ID. No. 1) an additional non-contiguous region of about 2,986 bp, XALB2 (Albicidin Biosynthetic Gene Cluster 2; SEQ ID. No. 2), and a third region of about 9,673 bp, XALB3 (Albicidin Biosynthetic Gene Cluster 3; SEQ ID. No. 3). Albicidin Biosynthetic Gene Clusters 1-3 may be referred to, collectively, as the Albicidin Biosynthetic Gene Clusters and these sequences were found to be required for biosynthesis of Albicidins. Homology analysis revealed the presence of (i) four large genes with a modular architecture characteristic of polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS) potentially involved in albicidin precursor biosynthesis; (ii) four

smaller genes potentially involved in albicidin substrate biosynthesis (iii) four modifying genes; (iv) one enzyme activating gene, (v) two regulatory genes, (vi) one chaperone gene, (vii) two genes of unknown function; and (viii) two resistance genes. These are named and discussed more fully below. Together these genes allow the successful operation of the biosynthetic pathway when cloned into suitable host cells.

[0088] Alignment of individual NRPS and PKS domains revealed an extraordinary biosynthetic apparatus believed to involve a *trans*-action of separate PKS and NRPS domains which could contribute to the production of multiple, structurally related albicidins by the same gene cluster. Furthermore, analysis of selectivity-conferring residues indicated that four NRPS modules of XALB1 specify an unusual substrate.

[0089] In an alternate embodiment the invention provides a method of producing a polyketide carrying para-aminobenzoic acid and/or carbamoyl benzoic acid by inserting at least one DNA fragment that encodes a PKS protein into a cell and causing the cell to express the encoded PKS protein under conditions such that the PKS protein functions to produce a polyketide carrying either a para-aminobenzoic acid or a carbamoyl benzoic acid or both. Another embodiment provides a method of producing polyketide/peptides carrying para-aminobenzoic acid and/or carbamoyl benzoic acid by inserting at least one DNA fragment that encodes a PKS protein into a cell and causing the cell to express the encoded PKS protein under conditions such that the PKS protein functions to produce a polyketide carrying either a para-aminobenzoic acid or a carbamoyl benzoic acid or both. In yet another embodiment, the invention provides a method of activating nonproteinogenic amino acids like paraaminobenzoic acid and/or carbamoyl benzoic acid for incorporation into peptides or polyketides by inserting at least one DNA fragment that encodes a PKS protein into a cell and causing the cell to express the encoded PKS protein under conditions such that the PKS protein functions to produce a polyketide carrying either a para-aminobenzoic acid or a carbamoyl benzoic acid or both.

[0090] There are three regions of the *X. albilineans* genome specifying albicidin production. XALB2 and XALB3 regions each contain only one gene, both of which are required for post-translational activation and folding of albicidin PKS and NRPS enzymes. The XALB1, XALB2 and XALB3 gene clusters are characterized by an unusual hybrid NRPS-PKS system, indicating that albicidin biosynthesis may provide an excellent model for investigating the biosynthesis of hybrid polyketide-polypeptide metabolites in bacteria. The availability of three genomic regions involved in albicidin production, XALB1 and XALB2 and XALB3, also offers the ability to express individually the enzymes of the albicidin family biosynthetic pathway including structural, resistance, secretory and regulatory elements, and to engineer overproduction of albicidin in mutated or modified host cells of the invention. The invention overcomes prior art limitations in albicidin production due to low yields of toxin production in *X. albilineans* and may

also allow characterization of the chemical structure of albicidin as well as application of this potent inhibitor of prokaryote DNA replication.

[0091] The invention results from a number of unpredictable results namely the number and complexity of the enzymes involved in biosynthesis. The discovery of the complete sequence required for biosynthesis of Albicidins is previously unreported. The invention provides for a novel process for production of molecule having a polyketide-polypeptide backbone and the formula $C_{40}H_{35}O_{15}N_6$, a molecular weight of 839, and the structural elements shown in Figure 11.

[0092] The invention further includes (a) the Albicidin Family Biosynthetic Gene Cluster including (b) the structural and regulatory elements of the operons that encode c) the enzymes PKS-1, PKS-2, PKS-3, PKS-4, NRPS-1, NRPS-2, NRPS-3, NRPS-4, NRPS-5, NRPS-6 and NRPS-7 as well as (d) the proteins AlbI to AlbXXII, (e) the isolated enzymes, proteins, and active forms thereof, as well as mutants, fragments, and fusion proteins comprising any of the forgoing; (f) the uses of the enzymes or proteins encoded by the Albicidins Biosynthesis Gene Cluster or any one of its operons, (g) a host cell expressing one or more enzymes or proteins encoded by the Albicidin Family Biosynthetic Gene Cluster; (h) use of host cells having the Albicidins Biosynthesis Gene Cluster to produce an antibiotic; (i) methods of modifying the DNA sequences to produce members of a series of antibiotic compounds having structures related to Albicidins; (j) DNA sequences that encode the same proteins as any of SEQ. ID. Nos. 1 to 25 but differ in specific codons due to the multiplicity of codons that lead to expression of the same amino acid; (k) antibiotics produced by the process of expression of the Albicidin Family Biosynthetic Genes in a genetically modified host cell sustained in a culture medium and thereafter separation of the antibiotic from the host cell and culture medium; (l) an isolated and purified antibiotic produced by a process that includes at least three proteins coded by DNA sequences selected for the group consisting of SEQ. ID Nos. 1 to 25 in combination with additional enzymes that modify the product to provide a non-naturally occurring Albicidins like product having at least one of the useful properties reported for albicidin; and (m) a process for producing an antibiotic that comprises modifying a host cell to enhance expression of the DNA of the Albicidin Family Biosynthetic Gene Cluster by insertion of expression enhancing DNA into the genome of a *Xanthomonas albilineans* strain in a position operative to enhance expression of the enzymes of the Albicidin Family Biosynthetic Gene Cluster, culturing the modified host cell to produce an antibiotic and isolating the antibiotic. The products and methods described above have utility as proteins or as nucleic acids as the case may be, including such uses sources of pyrimidine or purine bases or amino acids, or as animal food supplements and the like, as well as the more important uses to provide antibiotics, plant disease treatment methods, genetically modified disease resistant plants, phytotoxins and the like.

[0093] The subject invention also provides an isolated and purified antibiotic produced

by a process that includes at least three proteins coded by the nucleic acids of the subject invention in combination with additional enzymes that modify the product to provide a non-naturally occurring Albicidin-like product having at least one of the useful properties reported for albicidin. In certain embodiments, the antibiotic or antibiotics have at least one of the general structures illustrated in Figure 11. In other embodiments, antibiotics of the subject invention have at least 4 of the structural elements illustrated in Figure 11, and an elemental composition of $C_{40}H_{35}N_6O_{15}$.

[0094] The invention further provides a method of protecting a plant against damage from albicidin that comprises applying an agent that blocks expression at least one gene in the Albicidin Biosynthetic Gene Clusters to the plant to be protected. Additional inventions include a method of obtaining agents useful in blocking expression of albicidin by screening materials against a modified host cell line that expresses the Albicidin Biosynthesis Gene Clusters and selecting for materials that stop or decrease albicidin production and a method of protecting a plant against phytotoxic damage from an antibiotic that comprises inserting into the plant and operably expressing at least one resistance gene from the Albicidin Biosynthesis Gene Clusters into the plant to be protected.

EXAMPLE 1 – Materials and Methods

[0095] Bacterial strains and plasmids. The source of bacterial strains and their relevant characteristics are described in Table 1.

[0096] Media, antibiotics, and culture conditions. *X. albilineans* strains were routinely cultured on modified Wilbrink's (MW) medium at 30°C without benomyl (Rott *et al.*, 1994). For long-term storage, highly turbid distilled water suspensions of *X. albilineans* were supplemented with glycerol to 15% (vol/vol) and frozen at -80°C. For *X. albilineans*, MW medium was supplemented with the following antibiotics as required at the concentrations indicated: kanamycin, 10 or 25 µg/ml; and rifampicin, 50 µg/ml. *E. coli* strains were grown on Luria-Bertani (LB) agar or in LB broth at 37°C and were maintained and stored according to standard protocols (Sambrook *et al.*, 1989). For *E. coli*, LB medium was supplemented with the following antibiotics as required at the concentrations indicated: kanamycin, 50 µg/ml; ampicillin, 50 µg/ml.

[0097] Bacterial conjugation. DNA transfer between *E. coli* donor (DH5_MCR/pAlb389 or pAC389.1, Table 1) and rifampicin-resistant *X. albilineans* recipients (*X.* strains AM10, AM12, AM13, AM36 and AM37, Table 1) was accomplished by triparental conjugation with plasmid pRK2073 as the helper as described previously (Rott *et al.*, 1996).

[0098] Assay of albicidin production. Albicidin production was tested by a microbiological assay as described previously (Rott *et al.*, 1996). Rifampicin and kanamycin exconjugants were spotted with sterile toothpicks (2-mm-diameter spots) onto plates of SPA

medium (2% sucrose, 0.5% peptone, 1.5% agar) and incubated at 28° C for 2-5 days. The plates were then overlaid with a mixture of *E. coli* DH5 α (10⁷ cells in 2 ml of distilled water) plus 2 ml of molten 1.5% (wt/vol) Noble agar (Difco) at ca. 65° C and examined for inhibition zones after 24 h at 37°C.

[0099] Nucleic acid manipulations. Standard molecular techniques were used to manipulate DNA (Sambrook *et al.*, 1989) except for total genomic DNA preparation. Total genomic DNA for southern blot hybridization was prepared as described by Gabriel and De Feyter (1992).

[00100] PCR Conditions. PCR amplifications were performed in an automated thermal cycler PTC-100TM (MJ Research, Inc). The 25 μ l PCR reaction mix consisted of 100 ng of genomic DNA or 1 ng of plasmid DNA, 2.5 μ l of 10X PCR buffer without MgCl₂ (Eurobio), 80 μ M dNTP mix, 2.5 units of EUROBIOTAQII[®] (Eurobio), 25 pmoles of each primer, 2.0 mM MgCl₂ (Eurobio) and sterilized distilled water to final volume. The PCR program was 95°C for 2 min, 25 cycles at 94°C for 1 min, T_m for 1 min and 72°C for 1 min, with a final 72°C extension for 5 min. T_m temperature was determined for each couple of primers and varied between 55°C and 60°C. A 5 μ l aliquot of each amplified product was analyzed by electrophoresis through a 1% agarose gel. For sequencing, PCR products were cloned with the pGEM[®]-T Easy Vector System (Promega).

[00100] Oligonucleotide synthesis. Oligonucleotides were purchased from Genome Express (Grenoble or Montreuil, France).

[00101] DNA sequencing. Automated DNA sequencing was carried out on double-stranded DNA by the dideoxynucleotide chain termination (Sanger *et al.*, 1977) using a Dye Terminator Cycle Sequencing kit and an ABI Perkin-Elmer sequencer according to the manufacturer's procedure. Both DNA strands were sequenced with universal primers or with internal primers (20mers). This service was provided by Genome Express (Grenoble, France). Computer-aided sequence analyses were carried out using Sequence NavigatorTM (Applied Biosystems, Inc) and SeqMan (DNASTAR Inc.) programs.

[00102] Sequence analysis. Nucleotide sequences were translated in all six reading frames using EditSeq (DNASTAR Inc.). Potential products of ORFs longer than 100 b were compared to protein databases by the PSI-BLAST program (Swiss-Prot and Genbank) on the NCBI with site (ncbi.nlm.nih.gov/) using Altschul program (Altschul *et al.*, 1997). The TERMINATOR program of the Genetics Computer Group was used to identify putative Rho-independent transcription terminators.

[00103] Procedures**EXAMPLE 2 – Sequencing of the Double Strand Region of 55,839 Bp from *X. albilineans* Containing XALB1 SEQ ID NO. 1**

[00104] In Figure 1 is presented a physical map and genetic organization of XALB1. In the figure, E and K are restriction endonuclease sites for *EcoRI* and *KpnI*, respectively. Rectangular boxes represent DNA fragments labeled A through N. The numbers below each rectangular box are the number of Tn5-*gus* insertion sites previously located in each DNA fragment (Rott *et al.*, 1996). The DNA inserts carried by plasmids pALB571 and pALB540 are represented by bold bars above the physical map. The location and direction of putative orfs identified in the XALB1 gene cluster are shown by arrows. Precise positions and proposed functions for individual orfs are summarized in Tables 2 and 3, respectively. Position of insertional sites of eight albicidin-defective mutants determined by sequencing are indicated by vertical arrows. The location and direction of putative ORFS identified in the XALB1 gene cluster are shown by arrow shapes. These twenty putative ORFs are potentially organized in four or five operons, as indicated at the bottom of the figure. Patterns indicate NRPS and PKS genes (diagonal crosshatch), methyl transferase and esterase genes (hollow rectangles), carbamoyl transferase gene (fine crosshatch), benzoate-derived products biosynthesis genes (white), regulatory genes (vertical lined), resistance genes (diagonal lines) and other genes with function of unknown significance to albicidin production (black), and three insertional sites of eight albicidin-defective mutants determined by sequencing are indicated by vertical arrows. Dotted regions in the physical map and in ORFs represent the two internal duplicated DNA regions of XALB1.

[00105] The sequence illustrated in Figure 1 was generated as follows. The sources of DNA are set out in Table 1. DNA fragments F, E, B, C, I, and G, generated by the digestion of cosmid pALB571 (Rott *et al.*, 1996) with *EcoRI* and/or *KpnI*, were subcloned into pBCKS (+) and were sequenced from the resulting subclones, pBC/F, pBC/E, pBC/B, pBC/C, pBC/I and pBC/G. DNA fragment D' which corresponds to the part of fragment D present in cosmid pALB571 was sequenced from plasmid pUFR043/D' obtained following self ligation of the complete *EcoRI* digested cosmid pALB571. DNA fragment H was sequenced from pAM45.1 (Rott *et al.*, 1996), obtained following cloning into vector pBR325 of the 12kb *EcoRI* fragment carrying Tn5 and flanking sequences from mutant strain XaAM45. DNA fragment A' contains the part of fragment A present in cosmid pALB571 and was subcloned into vector pBCKS (+) and the resulting plasmid pBC/A' was used for sequencing. The presence of a large internal duplication made alignment of sequence data obtained from pBC/A' difficult. This difficulty was

resolved using sequence data obtained from an additional plasmid, pAM4, obtained following cloning into vector pBluescript II KS (+) of the 12kb *EcoRI* fragment carrying Tn5 and flanking sequences from mutant strain XaAM4, which contains only one copy of the large internal duplication. Sequence data from pBC/A' were used to determine the first 1542 bp of fragment A' between nucleotides C-19001 and G-20543. Sequence data from pAM4 and pBC/A' were used to determine the last 4823bp of fragment A' between nucleotides G-21653 and G-26477. The overlapping region between nucleotides G-20469 and C-22159 was amplified by PCR from cosmid pALB571 using primers contig13-1160 (5'gcgtaccgtgtccagtagg3') SEQ ID NO. 48 and pAM4-14 (5'gctggaaaccgagaatctga3') SEQ ID NO. 49, and was sequenced. Resulting sequence data were used to complete sequencing of DNA fragment A'. The junctions A/F, F/H, H/E, E/B, B/C, C/I, I/G, G/D between corresponding DNA fragments were sequenced directly from cosmid pALB571. *EcoRI* DNA fragment containing fragments A and F was subcloned from pALB540 into pBCKS (+), and the resulting plasmid pBC/AF was used to determine the part of DNA fragment A which was not present in cosmid pALB571 between nucleotides G-13682 and G-19001. *EcoRI* DNA fragments J, K, L, N were subcloned from pALB540 into pBCKS (+) and were sequenced from resulting plasmid pBC/J, pBC/K, pBC/L, and pBC/N. The junctions L/K, K/J and J/A between corresponding DNA fragments were sequenced directly from cosmid pALB540. DNA region between nucleotides G-7517 and T-8721 was amplified by PCR from cosmid pALB540 using primers E114 (5'gacacgatcagccgctagga3') SEQ ID NO. 50 and EI4-380 (5'accagcagttgggccagcct3') SEQ ID NO. 51 and was sequenced. Resulting sequence data were used to determine the sequence of fragment M and of junctions N/M and M/L. The nucleotide sequence of 55,839 bp containing the entire major gene cluster involved in Albicidin production was sequenced on both strands.

EXAMPLE 3 – Analysis of the Large Internal Duplications in the DNA Sequence of XALB1

[00106] The sequence of the 55,839 bp genomic region (SEQ ID NO. 1) contains two large internal duplications as shown by the dotted regions in the physical map of Figure 1. A direct duplication of 1736 bp was located in DNA fragment A between nucleotides G-19904 and G-21639 and between nucleotides G-23057 and G-24792. Another direct duplication of a 2727 bp was found in DNA fragments B and C between nucleotides C-40410 and G-43136 and between nucleotides C-46644 and G-49370. Comparison of the two copies of each duplication revealed that the two copies of the 1736 bp duplication are identical except for one nucleotide at position 21058, and that the two copies of the 2727 bp duplication are 98.8% identical and differ by 30 nucleotides.

EXAMPLE 4 – Comparison of XALB1 with the *xabB* *Eco*RI Fragment

[00107] Comparison of the DNA sequence of the 55,839 bp genomic region described in this study with the partial DNA sequence of 16,511 bp of the same region in Huang et al., 2001 (described by Huang et al. as an *Eco*RI fragment including full length *xabB* from *X. albilineans* strain Xa13 [GenBank accession No. AF239749]), revealed that the DNA sequence from strain Xa13 over 16,511 bp is identical to the sequence from strain Xa23R1, described herein, with the following exceptions: 1) five nucleotides are different at positions 42963, 42972, 42980, 43014 and 43071 of the XALB1 sequence, and 2) nucleotides from positions 43137 to 49370 are missing (internal to *albI*; refer Fig. 1). Analysis of genomic DNA of seven strains isolated from different countries (Australia, Reunion Island, Kenya, Zimbabwe and USA), digested by *Kpn*I and hybridized with the pBC/C plasmid (Table 1) labeled with ³²P, revealed that two DNA fragments corresponding to the XALB1 fragments B and C were present in all strains (data not shown). This result indicated that all studied strains contain *albI* and not *xabB* because in *albI* the pBC/C plasmid probe hybridizes with the large internal duplication present in both DNA fragments B and C (Figure 1). Based on this observation we postulated that the DNA sequence of *XabB* reported as full length by Birch in PCT WO 02/24736 A1 (Their seq. ID#1) appears to be incomplete and missing 6,234 bp of DNA sequence encoding 2,078 amino acids.

EXAMPLE 5 – Reading Frame Analysis in XALB1

[00108] Analysis of the 55,839 bp double strand region for coding sequences revealed the presence of 20 open reading frames (ORFs) designated *albI* to *albXX* (Table 2 below) which are distributed in four groups of genes according to their position and their orientation in the XALB1 cluster (Figure 1). Genes of each group may form part of the same operon as judged by their overlapping stop and start codons, or by the relatively short intergenic region which varies from 5 to 274 nucleotides. The 20 ORFs appear to be organized in four operons: operon 1 formed by *albI* - *albIV*; operon 2 by *albV* - *albIX*; operon 3 by *albX* - *albXVI*; operon 4 by *albXVII* - *albXX*. The majority of *alb* ORFs are initiated with an ATG codon, except *albI* and *albXVII* which are initiated with a TTG codon, and *albIV* and *albVI* which are initiated with a GTG start codon. In seven ORFs of XALB1, start codons are preceded by the consensus sequence GAGG which may correspond to the ribosome binding site. Other ORFs are preceded by a less conserved sequence which contain at least three nucleotides A or G and which may serve as a weak ribosome binding site.

EXAMPLE 6 – Sequencing of the Tn5 insertional site of eight tox⁻ mutants previously located in XALB1

[00109] Eight of the 45 *X. albilineans* Tox⁻ mutants complemented by cosmid pALB540 and/or cosmid pALB571 and previously described (Rott *et al.*, 1996) were further analyzed. All eight mutants contain a single Tn5 insertion and correspond to the following *X. albilineans* strains: XaAM7, XaAM15, XaAM45, and XaAM52 which are complemented by pALB571 but not by pALB540; XaAM4, XaAM29 and XaAM40 which are complemented by both cosmids; and XaAM1 which is complemented by pALB540 but not by pALB571. The Tn5 insertional site of each Tox⁻ mutant was sequenced from plasmids obtained following cloning in pBR325 or in pBluescript II KS (+) of the EcoRI fragments carrying Tn5 and flanking sequence using the sequencing primer GUSN (5'tgcccacaggccgctcgagt3') SEQ ID No. 52 that annealed 135 bp downstream from the insertional sequence IS50L of Tn5-*gusA*. The sequence of the Tn5 insertional site was compared with the 55,839 bp sequence containing XALB1 in order to determine the *alb* gene disrupted in each Tox⁻ mutant. *albI* is disrupted by the Tn5 insertion in XaAM15 and XaAM45 at position 33443 and 34229, respectively (Figure 1). *albIV* is disrupted by the Tn5 insertion in XaAM7 and XaAM52 at position 53704 and 53915, respectively. *albIX* is disrupted by the Tn5 insertion in XaAM4, XaAM29 and XaAM40 at position 21653, 23444 and 24376, respectively. *albXI* is disrupted by the Tn5 insertion in XaAM1 at position 13301. These results are in accordance with the previous characterization of Tox⁻ mutants using Southern blot hybridization (Rott *et al.*, 1996), except for XaAM1. The Tn5-*gusA* insertion site of XaAM1 was previously located in DNA fragment A (Rott *et al.*, 1996) but results of this study showed that this site is located in DNA fragment J (Figure 1).

EXAMPLE 7 – Homology analysis of proteins potentially encoded by XALB1

[00110] Preliminary functional assignments of individual ORFs were made by comparison of the deduced gene products with proteins of known functions in the Genbank database. The results are set out in Table 3 below. Among the ORFs identified from the sequenced XALB1 gene cluster, we found (i) four genes, *albI* SEQ ID No. 20, *albIV* SEQ ID No. 23, *albVII* SEQ ID No. 17 and *albIX* SEQ ID No. 15, encoding PKS and/or NRPS modules; (ii) one carbamoyl transferase gene, *albXV* SEQ ID No. 5; (iii) two esterase genes, *albXI* SEQ ID No. 9 and *albXIII* SEQ ID No. 7; (iv) two methyltransferase genes, *albII* SEQ ID No. 21 and *albVI* SEQ ID No. 18; (v) two benzoate-derived products biosynthesis genes, *albXVII* SEQ ID No. 11 and *albXX* SEQ ID No.14; (vi) two putative albicidin biosynthesis regulatory genes, *albIII* SEQ ID No. 22 and *albVIII* SEQ ID No. 16; (vii) two putative albicidin resistance genes, *albXIV* SEQ ID No. 6 and *albXIX* SEQ ID No. 13; and (viii) two additional ORFs encoding proteins similar to transposition proteins, *albV* SEQ ID No. 19 and *albXVI* SEQ ID No. 4. No known

function was found in the database for *albX* SEQ ID No. 10 and *albXII* SEQ ID No. 8. The potential product of *albXVIII* SEQ ID No. 12 appeared to be a truncation of an enzyme with strong similarity to 4-amino-4-deoxychorismate lyase and branched-chain amino acid aminotransferase. Since the gene encoding the predicted product is roughly half the length of other such lyase or aminotransferase genes, *albXVIII* may be the result of a recombination event and may be non functional.

EXAMPLE 8 – The *alb* PKS and/or NRPS genes

[00111] The potential product of *albI*, designated AlbI SEQ ID No. 20, is a protein of 6879 aa with a predicted size of 755.9 kDa. This protein is very similar to the potential product of the *xabB* gene from *X. albilineans* strain Xa13 from Australia (Huang *et al.*, 2001), but it differs in length and size (See Table 4 below). XabB is a protein of 4801 amino acids with a predicted size of 525.7 kDa. Comparison of AlbI with XabB revealed that the N-terminal regions from Met-1 to Ile-4325 of both proteins are identical except for five amino-acids which are Tyr-3941, Pro-3952, Ala-4054, Ala-4271 and Gln-4284 in AlbI and His-3941, Ala-3952, Val-4054, Val-4271 and Glu-4284 in XabB. The same comparison revealed that the AlbI C-terminal region from Arg-6404 to the stop codon is 100% identical to the XabB C-terminal region from Arg-4326 to the stop codon.

[00112] The N-terminal region (from Met-1 to Asp-3235) of AlbI is 100% identical to the corresponding region in XabB which was previously described as similar to many microbial modular PKS (Huang *et al.*, 2001). This PKS region may be divided into three modules (Figure 2). Abbreviations used in the Figure are: A, adenylation; ACP, acyl carrier protein; AL, acyl-CoA ligase; C, condensation; KR, β -ketoacyl reductase; KS, β -ketoacyl synthase; NRPS, nonribosomal peptide synthase; PCP, peptidyl carrier protein; PKS, polyketide synthase; TE, thioesterase; HBCL, 4-hydroxybenzoate-CoA ligase. The question mark in the NRPS-2 domain indicates that this A domain is incomplete. The first module designated PKS-1 contains acyl-CoA ligase (AL) and acyl carrier protein (ACP1) domains. The second module designated PKS-2 contains β -ketoacyl synthase (KS1) and β -ketoacyl reductase (KR) domains followed by two consecutive ACP domains (ACP2 and ACP3). The third module designated PKS-3 contains a KS domain (KS2) followed by a PCP domain (PCP1). Apart from their very high similarity with XabB, these three PKS modules exhibited the highest degree of overall similarity with polyketide synthases SafB and PksM from *Myxococcus xanthus* and *Bacillus subtilis*, respectively (Table 4). The motifs characteristic of these domains are 100% identical to those of XabB which were previously aligned with those from other organisms (Huang *et al.*, 2001). The AL domain contains the conserved adenylation core sequence (SGSSG) and the ATPase motif (TGD). The three ACP domains contain a 4'-phosphopantetheinyl-binding cofactor box GxDS(IL), except that

A replaced G in ACP1. Both KS domains contain motif GPxxxxxxxCSxSL around the active site Cys, and two His residues downstream from the active site Cys, in motifs characteristic of these enzymes. The KR domain contains the NAD(P)H-binding site GGxGxLG.

[00113] The PKS part of AlbI is linked by the PCP1 domain to the four apparent nonribosomal peptide synthase modules designated NRPS-1, NRPS-2, NRPS-3 and NRPS-4 (Figure 2). NRPS-1, NRPS-2 and NRPS-3 modules display the ordered condensation, adenylation (A) and PCP domains typical of such enzymes (Marahiel *et al.*, 1997), and NRPS-4 consists of an extra C domain which may correspond to an incomplete NRPS module. Known conserved sequences, characteristic of the domains commonly found in peptide synthases (Marahiel *et al.*, 1997), were compared to those from NRPS-1, NRPS-2, NRPS-3 and NRPS-4 (Tables 5, 6 and 7). Sequences characteristic of C, A, or PCP domains are conserved in these four NRPS, except in A domain of NRPS-2 module, suggesting that this latter A domain may be not functional. Comparison of the four NRPS modules among themselves revealed that NRPS-2, NRPS-3 and NRPS-4 modules were 30.7%, 94.4% and 47.5% similar to NRPS-1 module, respectively. Comparison with XabB revealed NRPS-2 and NRPS-3 modules were not present in XabB which contains only NRPS-1 and NRPS-4 modules (Figure 2). The dotted box in Figure 2 corresponds to the apparent deletion of the NRPS-2 and NRPS-3 modules in XabB as compared to AlbI. Apart their very high similarity with XabB, Alb I NRPS modules exhibited the highest degree of overall similarity with non-ribosomal peptide synthases NosA and NosC from *Nostoc* sp.

[00114] *albIV* potentially encodes a protein of 941 aa (AlbIV) with a predicted size of 104.8 kDa. AlbIV is similar to several non-ribosomal peptide synthases such as the BA3 peptide synthase involved in bacitracin biosynthesis in *Bacillus licheniformis* (Table 4). AlbIV forms one NRPS module designated NRPS-5 that contains only an A domain and a PCP domain (Figure 2). Sequences characteristic of the domains A and PCP commonly found in peptide synthases (Marahiel *et al.*, 1997) are conserved in AlbIV (Tables 6 and 7). However the A domain present in AlbIV differs from A domains commonly found in peptide synthases: conserved sequences corresponding to cores A8 and A9 in AlbIV are separated by a very long peptide sequence of 390 amino-acids. This additional peptide sequence exhibits a significant similarity with the protein WbpG of 377 amino acids involved in the biosynthesis of a lipopolysaccharide in *Pseudomonas aeruginosa* (Table 4).

[00115] *albVII* potentially encodes a protein of 765 aa (AlbVII) with a predicted size of 83.0 kDa similar to the 4-hydroxybenzoate-CoA ligase from several bacteria and the closest protein (HbaA) was from *Rhodopseudomonas palustris* (Table 4). High similarity between AlbVII and HbaA suggests that AlbVII is a 4-hydroxybenzoate-CoA ligase and constitutes a fourth PKS module designed PKS-4. The size of HbaA is smaller (539 aa) and the similarity between the two proteins starts only at the residue 277 of AlbVII and at the residue 28 of HbaA.

Comparison of AlbVII sequence located upstream from residue 277 produced no significant alignment. AlbVII, like 4-hydroxybenzoate-CoA ligases, contains some conserved sequences characteristic of the A domain commonly found in peptide synthases (Table 6).

[00116] *albIX* encodes a protein of 1959 aa (AlbIX) with a predicted size of 218.4 kDa similar to non-ribosomal peptide synthases. Known conserved sequences, characteristic of the domains commonly found in peptide synthases (Marahiel *et al.*, 1997), were compared with those from AlbIX which forms two NRPS modules designated NRPS-6 and NRPS-7 (Tables 5, 6 and 7). NRPS-6 contains only one A and one PCP domain. NRPS-7 contains the three domains characteristic of NRPS modules (A-C-PCP) followed by a TE domain (Figure 2). Apart their very high similarity with XabB, NRPS-6 and NRPS-7 modules exhibited the highest degree of overall similarity and identity with non-ribosomal peptide synthases Dhbf from *B. subtilis* and NosA from *Nostoc* sp. (Table 4).

EXAMPLE 9 – The alb carbamoyl transferase gene

[00117] *albXV* potentially encodes a protein of 584 aa with a predicted size of 65.2 kDa. This protein, AlbXV, is similar to BlmD, a carbamoyl transferase involved in bleomycin biosynthesis in *Streptomyces verticillus* (Du *et al.*, 2000), and to a probable carbamoyl transferase potentially expressed in *P. aeruginosa* (Table 4). High similarity of AlbXV with these proteins suggests that AlbXV is a carbamoyl transferase.

EXAMPLE 10 – The alb esterase genes

[00118] *albXI* potentially encodes a protein of 315 aa with a predicted size of 35.9 kDa. This protein, AlbXI, exhibits low similarity to SyrC, a putative thioesterase involved in syringomycin biosynthesis by *Pseudomonas syringae* (Zhang *et al.*, 1995), and to a potential hydrolase encoded by *Streptomyces coelicolor* (Table 4). Precise function of SyrC remains unknown but SyrC is similar to a number of thioesterases, including fatty acid thioesterases, haloperoxidases, and acyltransferases that contain a characteristic GxCxG motif. The corresponding SyrC domain GICAG is conserved in AlbXI which contains the sequence GWCQA, except that A replaces the last G, suggesting that AlbXI may be an esterase despite its low overall similarity with SyrC.

[00119] *albXIII* potentially encodes a protein of 317 aa with a predicted size of 34.5 kDa. This protein, AlbXIII, is similar to hypothetical proteins with unknown function from several bacteria including *Caulobacter crescentus* (Table 4). AlbXIII and these hypothetical proteins contain a GxSxG motif characteristic of serine esterases and thioesterases, the corresponding sequence in AlbXIII being GHSVG. In addition, AlbXIII presents a similarity with

the 2-acetyl-1-alkylglycerophosphocholine esterase which hydrolyzes the platelet-activating factor in *Canis familiaris* (Table 4), suggesting that AlbXIII is an esterase.

EXAMPLE 11 – The alb methyltransferase genes

[00120] *albII* potentially encodes a protein of 343 aa (AlbII) with a predicted size of 37.7 kDa. *albII* is 100% identical to the *xabC* cistron, previously described as encoding an *O*-methyltransferase downstream *xabB* (Huang *et al.*, 2000a). This conclusion is based on the similarity of XabC with a family of methyltransferases that utilize S-adenosyl-L-methionine (SAM) as a co-substrate for *O*-methylation including TcmO protein from *Streptomyces glaucescens* (Huang *et al.*, 2000a). AlbII contains three highly conserved motifs of SAM-dependent methyltransferases, including the motif I involved in SAM binding (Figure 3). In the Figure, identical or similar amino acids (A=G; D=E; I=L=V) are shown in bold. Numbers indicate the position of the amino acid from the N-terminus of the protein. Abbreviations used in the Figure are: Sgl-TcmO and Sgl-TcmN, multifunctional cyclase-hydratase-3-*O*-Mtase and tetracenomycin polyketide synthesis 8-*O*-Mtase of *Streptomyces glaucescens*, respectively (accession number: M80674); Smy-MdmC, midecamycin-*O*-Mtase of *Streptomyces mycarofaciens* (accession number: M93958); Mxa-SafC, Saframycin *O*-Mtase of *Myxococcus xanthus* (accession number: U24657); Ser-EryG, erythromycin biosynthesis *O*-Mtase of *Saccharopolyspora erythraea* (accession number: S18533); Spe-DauK, carminomycin 4-*O*-Mtase of *Streptomyces peucetius* (accession number: L13453); Sal-DmpM, *O*-demethylpuromycin-*O*-Mtase of *Streptomyces alboniger* (accession number: M74560); Shy-RapM, rapamycin *O*-Mtase of *Streptomyces hygroscopicus* (accession number: X86780); Sav-AveD, avermectin B 5-*O*-Mtase of *Streptomyces avermitilis* (accession number: G5921167); Sar-Cmet, mithramycin C-methyltransferase of *Streptomyces argillaceus* (accession number: AF077869); AlbII, putative albicidin biosynthesis C-Methyltransferase of *Xanthomonas albilineans* (SEQ ID No. 27); identical to XabC, accession number: AF239749).

[00121] Comparison of AlbII with the Genbank database revealed that AlbII, besides 100% identity to XabC, exhibited the highest degree of overall identity with MtmMII, a C-methyltransferase from *Streptomyces argillaceus* (Table 4) involved in C-methylation of the polyketide chain for mithramycin biosynthesis, suggesting that AlbII is a C-methyltransferase. XabC was not compared by Birch and co-workers with MtmMII (Huang *et al.*, 2000a) because the MtmMII sequence was not available until recently in the Genbank database. The three highly conserved motifs in SAM methyltransferases are also present in MtmMII (Figure 3), suggesting that AlbII is a C-methyltransferase SAM-dependent.

[00122] *albVI* potentially encodes a protein of 286 aa (AlbVI) with a predicted size of 32.1 kDa similar to several hypothetical protein from *Mycobacterium tuberculosis*

(Genbank accessions No. AAK46042, AAK48238, AAK44517, AAK46218) and from *S. coelicolor* (Genbank accession No. CAC03631). AlbVI is also similar to the tetracenomycin C synthesis protein (TcmP) of *Pasteurella multocida* (Table 4). Four highly conserved motifs in TcmP and other *O*-methyltransferases are also present in AlbVI (Figure 4), suggesting that AlbVI is an *O*-methyltransferase. In the Figure, identical or similar aa (A=G; D=E; I=L=V; K=R) are shown in bold. Numbers indicate the position of aa from the N-terminus of the protein. Abbreviations used in the Figure are: Sgl-tcmP, tetracenomycin C synthesis protein of *Streptomyces glaucescens* (accession number: C47127); Sme-PKS, putative polyketide synthase of *Sinorhizobium meliloti* (accession number: AAK65734); Pmu-tcmP: tetracenomycin C synthesis protein of *Pasteurella multocida* (accession number: AAK03406); Mtu-Omt: putative *O*-methyltransferase of *Mycobacterium tuberculosis* (accession number: AAK45444); Mlo-Hp: hypothetical protein containing similarity to *O*-methyltransferase of *Mesorhizobium loti* (accession number: BAB50127); Mtu-Hp1: hypothetical protein of *Mycobacterium tuberculosis* (accession number: AAK46042); Mtu-Hp2: hypothetical protein of *Mycobacterium tuberculosis* (accession number: AAK48238); Mtu-Hp3: hypothetical protein of *Mycobacterium tuberculosis* (accession number: AAK44517); AAK46218); Sco-Hp: hypothetical protein of *Streptomyces coelicolor* (accession number: CAC03631); AlbVI, putative albicidin biosynthesis *O*-Methyltransferase of *Xanthomonas albilineans* (this study). The three highly conserved motifs in SAM methyltransferases are not present in AlbVI, indicating that SAM is not a co-substrate of AlbVI.

EXAMPLE 12 – The alb derived-benzoate products biosynthesis genes

[00123] albXVII potentially encodes a protein of 716 aa with a predicted size of 79.8 kDa. This protein, AlbXVII, is very similar to the para-aminobenzoate (PABA) synthase from *Streptomyces griseus* (Table 4). This enzyme is required for the production of the antibiotic candicidin (Criado *et al.*, 1993).

[00124] albXVIII potentially encodes a protein of 137 aa with a predicted size of 15.0 kDa. This protein, AlbXVIII, is similar to the 4-amino-4-deoxychorismate lyase (ADCL) from *P. aeruginosa* (Table 4). The function of ADCL is to convert 4-amino-4-deoxychorismate into PABA and pyruvate. The length of AlbXVIII is smaller (Table 4) than the length of ADCL and the similarity of AlbXVIII with this protein starts only at residue 161. *albXVIII* is preceded by a small ORF encoding a sequence of 59 amino acids similar to the first 42 amino acids of ADCL from *P. aeruginosa*. These data suggest that *albXVIII* is probably a truncated form of *albXVIII* and probably not functional. *albXVIII* may, therefore, not be involved in albicidin biosynthesis. The region between *albXVII* and *albXVIII* was amplified by PCR from total DNA of *X. albilineans* Xa23R1 strain using primers ORFW (5'gcgagaggacaagctgctgc3') SEQ ID No. 53 and ORFY (5'cggttaggatgcagcgctcg3') SEQ ID No. 54 and was sequenced. Resulting sequence data

showed that the sequence of the PCR fragment was 100% identical to the sequence of pALB540, indicating that the recombination of *albXVIII* did not occur during cloning of the genomic fragment in pALB540.

[00125] *albXX* potentially encodes a protein of 202 aa with a predicted size of 22.6 kDa. This protein AlbXX is similar to the 4-hydroxybenzoate synthase potentially involved in ubiquinone biosynthesis by *Escherichia coli* (Siebert *et al.*, 1992).

EXAMPLE 13 – The alb regulatory genes

[00126] *albIII* potentially encodes a protein of 167 amino acids with a predicted size of 17.8 kDa that is similar to the transcription factors ComA of different bacteria such as *E. coli* and *B. licheniformis* (Table 4). ComA transcription factors appear to be involved in regulation of antibiotic production in bacteria. In *E. coli*, a gene similar to *comA* is present in the enterobactin biosynthesis gene cluster (Liu *et al.*, 1989). In *B. subtilis*, ComAB was described as a probable positive activator of lichenysin synthetase transcription (Yakimov *et al.*, 1998) and a gene similar to *comA* was shown to be essential for bacilysin biosynthesis (Yazgan *et al.*, 2001). These data suggest that AlbIII regulates transcription of genes involved in albicidin biosynthesis.

[00127] *albVIII* potentially encodes a protein of 330 aa with a predicted size of 37.7 kDa. This protein, AlbVIII, is very similar to the SyrP like protein from *S. verticillus* and to SyrP protein from *P. syringae* (Table 4). SyrP participates in a phosphorylation cascade controlling syringomycin synthesis (Zhang *et al.*, 1997) and the *syrP* like gene was described in the *S. verticillus* bleomycin biosynthetic gene cluster (Du *et al.*, 2000). These data suggest that AlbVIII regulates albicidin biosynthesis in *X. albilineans*.

EXAMPLE 14 – The alb resistance genes

[00128] *albXIV* potentially encodes a protein of 496 aa with a predicted size of 52.7 kDa. This protein, AlbXIV, is 100% identical to AlbF isolated from *X. albilineans* strain Xa13 (GenBank Accession AF403709; direct submission by Bostock and Birch and described as “a putative albicidin efflux pump which confers resistance to albicidin in *E. coli*”). AlbXIV and AlbF are closely related to a family of transmembrane transporters involved in antibiotic export and antibiotic resistance in many antibiotic-producing organisms. AlbXIV and AlbF exhibited the highest degree of overall identity with the putative transmembrane efflux protein from *S. coelicolor* (Table 4). These data suggest that AlbXIV and AlbF may be involved in albicidin resistance by transporting the toxin out of the bacterial cells that produce it. Alternatively, AlbXIV and AlbF may simply play a role in antibiotic secretion and/or plant pathogenesis to effect the transport of albicidin outside of producing cells.

[00129] *albXIX* potentially encodes a protein of 200 aa with a predicted size of 22.8 kDa. This protein, AlbXIX, is similar to the McbG protein from *E. coli* (Table 4). In *Enterobacteriae*, the McbG protein, together with two other proteins (McbE and McbF), was shown to cause immunity to the peptide antibiotic microcin B17 which inhibits DNA replication by induction of the SOS repair system (Garrido *et al.*, 1988). McbE and McbF proteins serve as a pump for the export of the active antibiotic from the cytoplasm, whereas a McbG alone also provides some protection: a well-characterized deficient-immunity phenotype is exhibited by microcin B17-producing cells in the absence of the immunity gene *mcbG* (Garrido *et al.*, 1988). The significant similarity between AlbXIX and McbG, together with the fact that albicidin also blocks DNA replication (Birch and Patil, 1985a) suggests that AlbXIX confers immunity against albicidin in *X. albilineans*.

EXAMPLE 15 – Transposition proteins

[00130] *albV* is 100% identical to the *thp* gene described in a divergent position upstream from *xabB* (Huang *et al.*, 2000a). The *thp* gene potentially encodes a protein of 239 aa displaying significant similarity to the IS21-like transposition helper proteins. In *X. albilineans* strain LS155 from Australia, insertional mutagenesis of *thp* blocked albicidin production, but *trans*-complementation failed, indicating the involvement in albicidin production of a downstream gene in the *thp* operon (Huang *et al.*, 2000a).

[00131] *albXVI* potentially encodes a protein of 88 aa with a predicted size of 9.8 kDa similar to the transposases from several bacteria such as *Xanthomonas axonopodis* or *Desulfovibrio vulgaris* (Table 4).

[00132] The presence of transposition proteins in the XALB1 cluster is probably a remnant from a past transposition event that may have contributed to the development of the albicidin XALB1 cluster.

EXAMPLE 16 – Unknown functions

[00133] *AlbX* potentially encodes a protein of 83 aa with a predicted size of 9.4 kDa. This protein, AlbX, is similar to an hypothetical protein from *P. aeruginosa* and to the MbtH protein from *Mycobacterium tuberculosis*. MbtH is a protein with unknown function found in the mycobactin gene cluster (Quadri *et al.*, 1998). A MbtH-like protein with unknown function was also described in the bleomycin biosynthetic gene cluster of *S. verticillus* (Du *et al.*, 2000). These data suggest that AlbX is involved in albicidin biosynthesis but its function remains unknown.

[00134] *albXII* potentially encodes a protein of 451 aa with a predicted size of 51.6 kDa. This protein, AlbXII, is very similar to a protein of 55 kDa encoded by the *boxB* gene in *Azoarcus evansii* (Table 4). This protein is a component of a multicomponent enzyme system

involved in the hydroxylation of benzoyl CoA, a step of aerobic benzoate metabolism in *Azoarcus evansii*, but its function remains unknown (Mohamed *et al.*, 2001).

EXAMPLE 17 – Prediction of amino acid specificity of Alb NRPS modules

[00135] In NRPSs, specificity is mainly controlled by A domains which select and load a particular amino-, hydroxy- or carboxy-acid unit (Marahiel *et al.*, 1997). The substrate-binding pocket of the phenylalanine adenylation (A) domain of the gramicidin S synthetase (GrsA) from *Brevibacillus brevis* was recently identified by crystal structure analysis as a stretch of about 100 amino acid residues between highly conserved motifs A4 and A5 (Conti *et al.*, 1997). Based on sequence analysis of known A domains, in relation to the crystal structure of the GrsA (Phe)substrate binding pocket, similar models have been published to predict the amino acid substrate which is recognized by an unknown NRPS A domain (Challis *et al.*, 2000; Stachelhaus *et al.*, 1999). These models postulate specificity-conferring codes for A domains of NRPS consisting of critical amino acid residues putatively involved in substrate specificity. The model proposed by Marahiel and co-workers (Stachelhaus *et al.*, 1999) defined a signature sequence consisting of ten amino acids lining with the ten residues of the phenylalanine-specific binding pocket located at positions 235, 236, 239, 278, 299, 301, 322, 330, 331 and 517 in the GsrA (Phe) sequence (accession number: P14687). The model proposed by Townsend and co-workers (Challis *et al.*, 2000) uses only the first eight of these critical residues.

[00136] Preliminary specificity assignments of albicidin synthase AlbI, AlbIV, AlbVII and AlbIX NRPS modules were made by comparison of complete sequences between conserved motifs A4 and A5 with sequences in the Genbank database. The corresponding sequence of the AlbIV NRPS-5 module is most related to domain 5 of bacitracin synthase 3 (BA3) from *B. licheniformis* that was suggested to activate Asn (Konz *et al.*, 1997). Corresponding sequences of AlbI and AlbIX NRPS-1, NRPS-3, NRPS-6 and NRPS-7 modules, apart from their very high similarity with XabB, exhibited the highest degree of overall identity (39%) with the Blm NRPS2 module of the biosynthetic gene cluster for bleomycin from *S. verticillus* that specifies for β -Alanine (Du *et al.*, 2000). The corresponding sequence of AlbVII PKS-4 produced the highest significant alignment with acetate-CoA ligase from *Sulfolobus solfataricus* (Genbank accession number: AAK41550), aryl-CoA ligase from *Comamonas testosteroni* (Genbank accession number: AAC38458) and 4-hydroxybenzoate-CoA ligase from *R. palustris*. The sequence between motifs A4 and A5 of the AlbI NRPS-2 could not be significantly aligned with any sequence present in the Genbank database. Comparison of this sequence with the corresponding sequence of GrsA (Phe) revealed that parts of the putative core and structural “anchor” sequences of AlbI NRPS-2 are deleted (Figure 5), suggesting that the AlbI NRPS-2 substrate binding pocket is not functional. In the Figure, amino acids of the six Alb NRPSs and of Alb PKS-4 that are identical or similar to GrsA or Blm sequences (A=G; D=E;

I=L=V; R=K) are shown in bold. Amino acids underlined in the GsrA sequence correspond to the phenylalanine-specific binding pocket. The positions of these amino acids in the GsrA primary sequence are indicated at the top of the figure. Amino acids underlined in the other sequences correspond to putative constituents of binding pockets, aligned with the seven residues of the phenylalanine-specific binding pocket of GsrA. Shaded amino-acids correspond to the putative core sequences and structural "anchors" based on comparison with the GsrA binding-pocket structure.

[00137] Alignment of the primary sequence between conserved motifs A4 and A5 of the AlbI, AlbIV, AlbVII and AlbIX NRPS-1, NRPS-3, NRPS-5, NRPS-6, NRPS-7 and PKS-4 modules with the corresponding sequence of GsrA (Phe) (Figure 5) revealed the putative constituents of binding pockets that constitute the codes as defined by Marahiel and co-workers (Stachelhaus *et al.*, 1999). These codes were compared with those of proteins most related to the sequence between the A4 and A5 motifs (Table 8) and were analyzed with the model proposed by Townsend and co-workers (Challis *et al.*, 2000, jhunix.hcf.jhu.edu/~ravel/nrps/). Using these codes, we were able to predict the Asparagine specificity of the AlbIV NRPS-5 module. The AlbIV NRPS-5 signature is 100% identical to BacC-M5 (Asn) and TyrC-M1 (Asn) codes identified in bacitracin synthetase 3 from *B. licheniformis* and in tyrocidine synthetase 3 from *B. brevis* (Table 8). The AlbIV NRPS-5 signature is also identical to the Asn code defined by Marahiel and co-workers (1997), except that I is replaced by L at position 299 (Table 8). The AlbI and AlbIX NRPS-1, 3, 6 and 7 signatures did not match any of those defined by Marahiel and co-workers (1997). Similarly, convincing predictions using the model proposed by Townsend and co-workers were not obtained either (Challis *et al.*, 2000, jhunix.hcf.jhu.edu/~ravel/nrps/). The AlbI and AlbIX NRPS-1, 3, 6 and 7 signatures diverged from all NRPS signatures previously described, except from the XabB signature that is identical to the AlbI NRPS-1 and 3 signatures. The signature most closely related to AlbI NRPS-1 and 3 specify Pro and the signature most closely related to AlbIX NRPS-6 and 7 specify Ser, but the degree of similarity in both cases is very weak (Table 8). The PKS-4 signature is similar to the AlbI NRPS-1 and NRPS-3 signatures at positions 235, 299 and 301.

[00138] Analysis of alignment of the primary sequence between conserved motifs A4 and A5 of the AlbI and AlbIX NRPS-1, NRPS-3, NRPS-6 and NRPS-7 modules with the corresponding sequences of the bleomycin synthase (Blm) NRPS2 (β -Ala) and gramicidin S synthetase (GsrA) modules (Figure 5) revealed that (i) sequences of AlbI NRPS-1 and AlbI NRPS-3 differ only at the level of two residues that are not involved in substrate binding, (ii) sequences of AlbIX NRPS-6 and AlbIX NRPS-7 are 100% identical, (iii) sequences of AlbI NRPS-1 and AlbI NRPS-3 are very similar to sequences of AlbIX NRPS-6 and AlbIX NRPS-7 but differ at the level of five putative constituents of binding pocket, (iv) AlbI and AlbIX NRPS residues, which are similar to residues of Blm NRPS2 (β -Ala) or GsrA (Phe), are essentially

located at the level of the putative core sequences and structural “anchor”, and differ at the level of putative constituents of the binding pocket.

[00139] Binding-pocket constituents forming the NRPS signatures have been classified into three subgroups according to their variability among 160 specificity-conferring signature sequences (Stachelhaus *et al.*, 1999): (i) invariant residues Asp235 and Lys517 that mediate key interactions with the α -amino and α -carboxylate group of the substrate, respectively; (ii) moderately variant residues in positions 236, 301 and 330 which correspond to aliphatic amino acids and which may modulate the catalytic activity and fine-tune the specificity of the corresponding domains; (iii) highly variant residues in positions 239, 278, 299, 322 and 331 which may facilitate substrate specificity. AlbI and AlbIX NRPS-1, 3, 6 and 7 signatures are not totally in accordance with this classification. Invariant residue Lys517 is conserved in the four NRPS signatures, indicating the presence of an α -carboxylate group in the corresponding substrates. The Asp235Ala alteration is not consistent with an α -amino acid substrate. Birch and co-workers (Huang *et al.*, 2001) assumed that the initial alanine residue in the XabB signature was consistent with a nonproteinogenic hydroxy acid substrate by analogy with the initial glycine in the signature of the hydroxyisovaleric-acid (HVCL) loading domain of enniatin synthetase. The presence of an initial Alanine in the AlbVII PKS-4 signature (Figure 8) and in several 4-hydroxybenzoate-CoA ligase codes may confirm this hypothesis. However, the HVCL loading domain of enniatin synthetase (Table 8) and AlbVII PKS-4 are not preceded by a C domain and are not followed by a PCP domain, in contrast to the AlbI and AlbIX NRPS-1, 3, 6 and 7 modules. An Asp235Val alteration was recently described in the β -Ala specificity-conferring code (Du *et al.*, 2000, Table 8), suggesting that the substrate of AlbI and AlbIX NRPS-1, 3, 6 and 7 modules may be different from α -amino acids but may contain an amino group. Residue 236 is an aliphatic residue (Val or Ile) in all AlbI and AlbIX NRPS-1, 3, 6 and 7 signatures. Residue 301 is an aliphatic residue (Ala) in the AlbI NRPS-1 and 3 codes, but it is a Ser in the AlbIX NRPS-6 and 7 signatures. Residue 330 is not an aliphatic residue in the four NRPS signatures but an Asp. Similar alterations are present in the β -Ala code: residue 236 is an Asp, residue 301 is a Ser and residue 330 is an aliphatic amino acid. Concerning highly variable residues, AlbI NRPS-1 and 3 signatures differ from AlbIX NRPS-6 and 7 signatures at residue positions 299, 322 and 331, confirming that both types of NRPS modules specify different substrates.

[00140] **Table 8 : Comparison of signature sequences, as defined by Marahiel and co-workers** (Stachelhaus *et al.*, 1999), derived from sequences between the A4 and A5 domains of the AlbI, AlbIV, and AlbIX NRPS modules with those of Tyr-M1 (Pro) (Tyrocidine synthetase 2 module 1, accession number: AAC45929), VirS (Pro) (Virginiamycin S synthetase, accession number : CAA72310), HVCL (hydroxyisovaleric acid-CoA ligase, ACL1 enniatin synthetase, accession number: S39842), EntF-M1 (Ser) (Enterobactin synthase, accession number: AAA92015), β -Ala code (β -Ala selectivity-conferring code defined by Du *et al.*, 2000),

BacC-M5 (Asn) (Bacitracin synthetase 3, accession number: AAC06348), TyrC-M1 (Asn) (Tyrocidine synthetase 3, accession number: AAC45930) and Asn code (Asn selectivity-conferring code defined by Marahiel and co-workers (Stachelhaus *et al.*, 1999). Amino acids of AlbI NRPS-1 and NRPS-3 signatures identical or similar to TyrB-M1 (Pro), VirS (Pro) and HVCL signatures (A=G; D=E; I=L=V; R=K) are shown in bold. Amino acids of AlbIX NRPS-6 and NRPS-7 signatures identical or similar to Vir (Pro) and Blm (β -Ala) signatures (A=G; D=E; I=L=V; R=K) are shown in bold. Variability: 0 indicates invariant residues, +/- moderately variant residues and ++ highly variant residues.

EXAMPLE 18 – Identification of putative promoters and putative terminators in XALB1

[00141] Putative rho independent terminators were identified downstream from *albIV* and *albXVI* using the Terminator program (Brendel and Trifonov, 1984), run with the Wisconsin PackageTM GCG software (Figure 6). In the Figure, dashes indicate palindromic sequences. Symbols used in the Figure are: P, Primary structure value of putative terminator (minimum threshold value of 3.5 represents 95 percent of known, factor-independent, prokaryotic terminators); S, Secondary structure value of putative terminator. The presence of these terminators confirmed the proposed genetic organization of operons 1 and 3. A rho-independent terminator was identified in the intergenic region between *albXVII* and *albXVIII*, suggesting that the group of genes initially supposed to be organized in operon 4 may be in fact organized in two operons, operon 4 formed by *albXVII* and operon 5 by *albXVIII* B *albXX*. No putative rho independent terminator was found downstream from *albIX* and from *albXX*.

[00142] The 236 bp region between *albI* (operon 1) and *albV* (operon 2) is 100% identical to the sequence between *xabB* and *thp* genes that is assumed to contain a bidirectional promoter (Huang *et al.*, 2000a and 2001), suggesting that transcription of operon 1 and 2 is regulated by the same bidirectional promoter region (Huang *et al.*, 2001).

[00143] The 412 bp region comprised between *albX* (operon 3) and *albXVII* (operon 4) also contains a putative bidirectional promoter (Figure 7). In the Figure, the sequence of putative promoters are underlined, and putative ATG or TTG start codons are in bold. The closest matches (TTGACA-18x-TATAGT) to the consensus -35 (TTGACA) and -10 (TATAAT) sequences for *E. coli* σ^{70} promoters occurs 61 bp upstream from *albX* (operon 3). The closest matches (TTCAGA-19x-TATACA) to the consensus sequences for *E. coli* σ^{70} promoters occur 320 bp upstream from *albXVII* (operon 4). The region between *albXVII* and *albXVIII* lacks any apparent *E. coli* σ^{70} promoter. However, the sequence immediately upstream from *albXIX*, corresponding to the coding sequence of *albXVIII*, potentially contains an unidirectional promoter (Figure 7). The closest match (TTGCTC-19x-TATATT) to the consensus sequences for *E. coli* σ

⁷⁰ promoters occurs 33bp upstream from *albXIX*. The presence of a terminator downstream from *albXVII* and of a promoter upstream from *albXIX* suggests that *albXVIII* is not transcribed and that *albXIX* and *albXX* form operon 5.

EXAMPLE 19 – Cloning of the XALB2 gene cluster

[00144] The 6 kb *EcoR* I fragment carrying Tn5 and flanking sequence from strain AM37 was cloned in pBR325 and the obtained plasmid was designated pAM37 (Table 1). A 1.1 kb *Hind* III-*Hind* III DNA fragment from pAM37, named PR37 (Table 1), was labeled with ³²P and used to probe the 845 clones from the genomic library of *X. albilineans* strain Xa23R1, previously described (Rott et al., 1996). Eight new cosmids hybridized to this probe and restored albicidin production in mutant AM37. One of these cosmid, pALB389, carrying an insert of about 37 kb (Table 1), was used for complementation studies of the five mutants not complemented by pALB540 and pALB571. Cosmid pALB389 complemented mutants AM10 and AM37. Mutant AM10 was initially thought to be complemented by pALB639 (Rott et al., 1996). However, further complementation studies showed that mutant AM10 was not complemented by pALB639 and that only three mutants (AM12, AM13 and AM36) were complemented by pALB639 containing the third genomic region XALB3 involved in albicidin production. A 3 kb *EcoRI*-*EcoRI* DNA fragment from pALB389 that hybridized with probe PR37 was sub-cloned in pUFR043 (Table 1). The resulting plasmid pAC389.1 complemented mutants AM10 and AM37, confirming that the second region involved in albicidin production, XALB2, was present in the 3 kb insert of pAC389.1.

EXAMPLE 20 – Cloning of the XALB3 gene cluster

[00145] Cosmid pALB639, carrying an insert of 36 kb (Rott et al., 1996; Table 1) was used as a probe to compare the *EcoRI* restriction profiles of *X. albilineans* strain Xa23R1 with those of mutants AM12, AM13 and AM36 which were supposed to be mutated in the XALB3 gene cluster. An 11 kb band which was found in strain Xa23R1 but not in the three mutants was supposed to contain the XALB3 gene cluster. A 9.7 kb *EcoRI* DNA fragment purified from cosmid pALB639 also used as a probe in Southern blot analyse revealed the same 11 kb band. This 9.7 kb *EcoRI* DNA fragment was sub-cloned in pUFR043 (Table 1) and the resulting plasmid pAlb639A complemented mutants AM12, AM13 and AM36. The third region involved in albicidin production, XALB3, was therefore present in the 9.7 kb insert of pAlb639A.

EXAMPLE 21 – Sequencing of the Tn5 insertional site of *tox*^I mutants located in XALB2 and XALB3 and sequencing of the genomic regions XALB2 and XALB3

[00146] In Figure 8, E, H, Sa and S indicate restriction endonuclease cut sites for *EcoRI*, *HindIII*, *SalI* and *Sau3AI*, respectively. The DNA inserts carried by plasmids pAC389.1, pALB639A or pEV639 are represented by the bars at the top of the respective figures. Positions of the Tn5 insertional sites of mutants AM10, AM12, AM36 and AM37 were determined by sequencing and are indicated by vertical arrows. The DNA region corresponding to the Tn5 flanking regions in pAM10, pAM12.1, pAM36.2 and pAM37 and in the PR37 DNA fragment are represented by the bars at the bottom of the respective figures. The location and direction of *albXXI* and *albXXII* are indicated by thick black arrows. The location of other orfs in XALB2 similar to those described by Huang *et al.* (2000b) are indicated by thick white arrows.

[00147] The 7 kb *EcoR* I fragment carrying Tn5 and flanking sequence from strain AM10 was cloned in pBluescript II KS (+), and the obtained plasmid was designated pAM10 (Table 1). The sequences between *EcoRI* sites and the Tn5 insertional site of mutants AM10 and AM37 were sequenced from the resulting plasmids pAM10 and pAM37, respectively. The complete double-strand nucleotide sequence of the 2,986 bp *EcoR* I B *EcoR* I insert of pAC389.1 was determined from sequencing results of plasmids pAC389.1, pAM10 and pAM37 (Figure 8). The Tn5 insertional sites of mutants AM10 and AM37 were sequenced from plasmids pAM10 and pAM37 (Table 1), respectively, using the sequencing primer GUSN (5'tgcccacaggccgctcgagt3') that annealed 135 bp downstream from the insertional sequence IS50L of Tn5-*gusA*. The Tn5 insertional site of AM10 and AM37 was located at position 2107 and 1882, respectively.

[00148] The *EcoRI* fragments carrying Tn5 and the flanking sequences from mutants AM12 and AM36 were cloned in pBR325 (Rott *et al.*, 1996; Table1). The sequences between *EcoRI* site and the Tn5 insertional site of mutants AM12 and AM36 were sequenced from the resulting plasmids pAM12.1 and pAM36.2, respectively. The complete double-strand nucleotide sequence of the 9,673 bp *EcoR* I B *Sau3A* I insert of pALB639A was determined from the sequencing results of plasmids pAM12.1, pAM36.2 and pALB639A (Figure 8). The Tn5 insertional site of mutants AM12 and AM36 was sequenced from plasmids pAM12.1, pAM36.2 using the sequencing primer GUSN (5'tgcccacaggccgctcgagt3') that annealed 135 bp downstream from the insertional sequence IS50L of Tn5-*gusA*. The Tn5 insertional site of AM12 and AM36 was located at position 6500 and 7232, respectively (Figure 8).

EXAMPLE 22 – Homology analysis and genetic organization of XALB2 (Figure 8).

[00149] The sequence of 2986 bp containing XALB2 is 99.4% identical to the sequence of 2989 bp containing *xabA* described in *X. albilineans* strain LS155 from Australia (Huang et al., 2000b; accession number AF191324). The Tn5 insertional site of mutant LS156 described in *xabA* is 15 bp upstream from the insertional site of AM37. The orf disrupted in AM37 and AM10, designed albXXI, is identical to *xabA* except a C which replaces a T at position 1642. albXXI potentially encodes a protein of 278 aa with a predicted size of 29.3 kDa which is 100% identical to the potential product of *xabA*, described as a phosphopantetheinyl transferase (Huang et al., 2000b). Region XALB2 contains three additional orfs (orf1, orf2, and orf3) similar to those described by Huang et al., (2000b; orf, *rsp6* and *aspT*). orf2 and orf3 are 100% identical to *rsp6* and *aspT* respectively, and orf1 is similar to but smaller than orf. There are no close matches to the *E. coli* $\gamma 70$ promoter B10 (TATAAT) and B35 (TTGACA) consensus sequence, and no putative RBS site upstream from the putative start codon ATG of albXXI. The putative factor-independent transcription site described at 42 bp downstream from the TGA stop codon of *xabA* (Huang et al., 2000b) is also present at the same position downstream from albXXI.

EXAMPLE 23 – Homology analysis and genetic organization of XALB3 (Figure 8).

[00150] The orf disrupted in mutants AM12 and AM36 was located between nucleotide 6090 (ATG) and 8009 (TAA) and was designed *albXXII*. The first ATG at position 6090 is not preceded by a putative ribosome binding sequence, suggesting that the start codon is the ATG at position 6105 which is preceded at position B9 by the putative ribosome binding site sequence GGAG. A putative rho independent terminator was identified at position 8082, 73 b downstream from *albXXII* (Figure 6). There are no close matches to *E. coli* σ^{70} promoter B10 (TATAAT) and B35 (TTGACA) consensus sequence upstream from the putative start codon. The *SaII* DNA fragment corresponding to DNA sequence from nucleotide 5510 to nucleotide 8124, which contains the 595 bp upstream from the putative start codon, the orf *albXXII* and the putative rho independent terminator, was sub-cloned in pUFR043 in the opposite direction to LacZ (Table 1). The resulting plasmid pEV639 (Table 1) complemented mutants AM12, AM13 and AM36, confirming that (i) the third region involved in albicidin production, XALB3, was present in the insert of pEV639; (ii) *albXXII* is not transcribed as part of a larger operon; and (iii) the 595 bp upstream the putative start codon contain a promoter.

[00151] The potential product of *albXXII*, designated AlbXXII, is a protein of 634 aa with a predicted size of 71.5 kDa. This protein is very similar to the heat shock protein HtpG from *Pseudomonas aeruginosa* (identities = 82%) and from *Escherichia coli* (identities = 60%) (Table 4). The methionine encoded by the putative start codon at position 6105 was aligned with

the first aminoacid of the heat shock protein HtpG from *Pseudomonas aeruginosa*, confirming that *albXXII* initiates at position 6105.

Complementation of Tox⁻ mutants with the *albXXII* gene in fusion with LacZ

[00152] A 1,948 bp fragment corresponding to the entire 1,903 bp orf of *albXXII* and flanking nucleotides was PCR amplified from cosmid pALB639 with the forward primer 5'ttggaattcgacactaccgatgagcgtgg3' and the reverse primer 5'ttggatccgtgcgtcactgcttacgccg3'. Convenient in frame-*EcoRI* and *BamHI* restriction sites for further cloning were simultaneously introduced with forward and reverse PCR primers, respectively. The PCR fragment was cloned into pGEMT vector (Promega) and sequenced. Several clones of the resulting plasmid pGemT/*albXXII* were sequenced. Because several PCR derived point mutations were observed in all the sequenced clones, a 1,920 bp *BglIII* – *SalI* fragment from pEV639 (corresponding to the 1,809 5' terminal nucleotides of *albXXII* orf plus 111 bp downstream the stop codon) was cloned into a pGemT/*albXXII* clone between the *BglIII* site located at position 94 of the *albXXII* orf and the *SalI* site of the vector's multiple cloning site. The resulting plasmid pGemT/*albXXIIbis* contained an intact *albXXII* orf that was then subcloned as an *EcoRI* – *SalI* fragment into pUFR043 to generate pEValbXXII. This construct of *albXXII* in fusion with LacZ was transferred by triparental conjugation into Xa23RI insertion mutants. pEValbXXII complemented mutants AM12, AM13 and AM36 (see table 9). These results confirmed that (i) the third region involved in albicidin production, XALB3, was present in the insert of pEValbXXII; and (ii) *albXXII* is not transcribed as a part of a larger operon.

Complementation of Tox⁻ mutants with the *htpG* gene from *E. coli*

[00153] A 2,343bp fragment corresponding to the *htpG* gene of *E. coli* plus 458 bp downstream the stop codon was PCR amplified from purified DH5 α genomic DNA with forward primer 5'ttggaattccatgaaaggacaagaaactcgtgg3' and reverse primer 5'gcctgcggaatggtacgcgggaagccgtcc3'. A convenient in frame-*EcoRI* restriction site was introduced with the forward PCR primer. The PCR fragment was cloned using the pGEMT vector system (Promega). Three resulting clones potentially containing plasmid pGemT/*HtpG* were sequenced, and one clone containing the correct sequence was selected. The 2,343bp PCR insert was then subcloned as an *EcoRI* – *SalI* fragment into pUFR043 to generate pEVHtpG, the *SalI* site corresponding to the site of the vector's multiple cloning site. This *HtpG* gene, in fusion with the LacZ construct, was able to restore albicidin production after transfer by triparental conjugation into AM12, AM13 and AM36 Xa23RI mutants. This result is i/ further evidence of the involvement of a molecular chaperone HtpG in the biosynthesis of albicidin (table 9), ii/ the first report of the requirement of a molecular chaperone HtpG in NRPS and PKS metabolism.

EXAMPLE 24 - Heterologous production of albicidin in fast growing *Xanthomonas axonopodis* pv. *Vesicatoria*.

[00154] This example illustrates the construction of a heterologous expression system harboring the three XALB regions, its transfer into a fast growing host, *Xanthomonas axonopodis* pv. *vesicatoria* and the subsequent production of a potent toxin with an antibiotic activity similar to that of albicidin. This work is a milestone in the validation of the albicidin biosynthesis model because it gives experimental evidence that the entire biosynthetic machinery required for albicidin biosynthesis has been identified, cloned, sequenced and transferred into an heterologous host, driving the production of albicidin. Cosmid pALB571 which covers the complete sequences of operons 1 and 2 was used to transfer operons 1 and 2 (Figure 1). Operons 3 and 4 (from pALB540), XALB2 (from pAC389.1) and XALB3 (from pEV639) were subcloned into a single plasmid, pOp3-4/XALB2-3 (see below). Plasmid pOp3-4/XALB2-3 derived from shuttle vector pLAFR3 that carries one selective gene for resistance to tetracyclin and that belongs to incompatibility group IncP (Table 1). Cosmid pALB571 derived from shuttle vector pUFR043 that carries two selective genes for resistance to kanamycine and gentamycine and that belongs to incompatibility group IncW (Table 1).

Sub-cloning of operons 3 and 4 and XALB2 and XALB3 regions into a single plasmid (Figure 12).

[00155] A 2,787 bp *Bam*HI – *Pst*II fragment from pALB540, corresponding to a portion of operon 4, was subcloned into pBCKS(+), yielding pBC/Op4Δ (step 1). A *Xho*I site was introduced into this vector immediately upstream from the *Bfr*I site by directed mutagenesis. Mutagenesis was performed with primers *Xho*IAIb anticodant 5'cgccctaagcagctcgagtagactgcaatc3' and *Xho*IAIbcodant 5'gattgcagtctactcgagctgcttaaggcg3' and yielded plasmid pBC/Op4Δ*Xho*I (step 2). The 2,986 bp *Eco*RI fragment from pAC389.1 (containing XALB2) was then subcloned into pBC/Op4Δ*Xho*I, yielding pBC/Op4Δ/XALB2 (step 3). A 10,762 bp *Bfr*I fragment from pALB540 and containing complete operon 3 and the beginning of operon 4 was subcloned into pBC/Op4Δ/XALB2 yielding pBC/Op3-4/XALB2 (step 4). The 2,615 bp *Sal*I fragment from pEV639 (containing XALB3) was subcloned into pBKS, yielding pBKS/XALB3 (step 5). The *Sal*I site located on the *Kpn*I side of the polylinker was then destroyed and substituted by a *Xho*I restriction site by directed mutagenesis. This mutagenesis was performed with primers *Xho*SalXaHTPGR 5'gcttatcgataccctcgaggaaggcgatatcg3' and *Xho*SalXaHTPGF 5'cgatatcgccctcctcgagggtatcgataagc3', yielding pBKS/XALB3*Xho*I (step 6). Finally, the *Xho*I cassette of pBC/Op3-4/XALB2 was subcloned into the *Sal*I restriction site of pBKS/XALB3*Xho*I, yielding pBKS/Op3-4/XALB2-3 (step 7). This construct harbours an *Xho*I cassette containing complete operons 3 and 4 from XALB1, *albXXI* from XALB2 and *albXXII*

from XALB3. An *Xho*I site was added to the *Bam*HI site of the pLAFR3 shuttle vector polylinker using the adaptor AdApTBamHIXhoI 5' gatcgctcgagc3', yielding pLAFR3XhoI (step 8). The *Xho*I cassette from pBKS/Op3-4/XALB2-3 was then cloned into pLAFR3XhoI, yielding pOp3-4/XALB2-3 (step 9). This last construct was used, along with pALB571 (operons 1 and 2), for heterologous expression of albicidin in *X. axonopodis* pv. *vesicatoria*.

Albicidin production assays

[00156] The four combinations of plasmids (i.e. pUFR043-pLAFR3, pUFR043-pOp3-4/XALB2-3, pAlb571-pLAFR3 and pAlb571-pOp3-4/XALB2-3) were transferred into *X. axonopodis* pv. *vesicatoria* strain Xcv 91-11BR1 by triparental mating. Exconjugant clones resistant to tetracycline and kanamycin were isolated. Assays for albicidin production were performed with these exconjugants clones using the same method described in Example 1 except that tetracycline (12 mg/ml) and/or kanamycin (50 mg/ml) were added to SPA medium. Tetracycline and kanamycin resistant *E. coli* clones, DH5 α KT and DH5 α Alb^rKT (Table 1), were used as tester strains to evaluate albicidin production to ensure that growth inhibition was not due to the presence of these two antibiotics in SPA medium. Both clones, DH5 α KT and DH5 α Alb^rKT, are tetracycline and kanamycin resistant because they carry plasmids pLAFR3 and pUFR043. The albicidin resistant DH5 α Alb^rKT clone derived from strain DH5 α Alb^r (Table 1) which is a spontaneous albicidin resistant clone isolated in a growth inhibition zone produced by *X. albilineans* strain Xa23R1.

[00157] Without antibiotics in the SPA medium, growth of clones DH5 α KT and DH5 α Alb^rKT was not inhibited in all assays performed with the different *X. axonopodis* pv. *vesicatoria* exconjugants. Surprisingly, when kanamycin was present in the SPA medium, growth of both DH5 α KT and DH5 α Alb^rKT was inhibited in all assays performed with the *X. axonopodis* pv. *vesicatoria* exconjugants. These results suggested that, in the presence of kanamycin, all *X. axonopodis* pv. *vesicatoria* exconjugants produced an antibiotic inhibiting growth of *E. coli*. Because exconjugants containing only empty vectors (pUFR043 and pLAFR3) induced inhibition of *E. coli*, this antibiotic did not result from the expression of XALB1, XALB2 and/or XALB3. Additionally, there was no cross resistance between this antibiotic and albicidin. When tetracycline was present in the bioassay medium, but not kanamycin, growth of the albicidin resistant clone (DH5 α Alb^rKT) was not inhibited by any of the exconjugants. In contrast, growth of the albicidin susceptible *E. coli* strain (DH5 α KT) was inhibited by the exconjugants harbouring pALB571 and pOp3-4/XALB2-3 plasmids, but not by exconjugants harbouring the other three combinations of plasmids (Table 10). This result suggested that expression of the XALB1, XALB2 and XALB3 regions in *X. axonopodis* pv. *vesicatoria* (harbouring pALB571 and pOp3-4/XALB2-3 plasmids) led to the production of an albicidin-like antibiotic. This product inhibited growth of an albicidin sensitive *E. coli* (DH5 α KT) and had no effect on the growth of an albicidin resistant clone

(DH5 α Alb⁺KT).

[00158] Preliminary results indicated that pLAFR3 derived plasmids were relatively unstable in the absence of tetracycline in the culture medium, suggesting that genes carried by pOp3-4/XALB2-3 were not expressed when *X. axonopodis* pv. *vesicatoria* exconjugants pALB571/pOp3-4/XALB2-3 were grown without tetracycline. Consequently, these exconjugants did not produce the albicidin-like compound in absence of any antibiotic in the culture medium (Table 10). Preliminary results also indicated that pUFR043 derived plasmids are relatively stable in *X. axonopodis* pv. *vesicatoria* in absence of antibiotic selection, suggesting that genes carried by pALB571 are expressed when *X. axonopodis* pv. *vesicatoria* exconjugants pALB571/pOp3-4/XALB2-3 were grown on media without kanamycin. Consequently, these exconjugants produced the albicidin-like compound on SPA containing only tetracycline.

[00159] Two *E. coli* DH5 α KT clones, that spontaneously grew within the growth inhibition zone of a *X. axonopodis* pv. *vesicatoria* pALB571-pOp3-4/XALB2-3 exconjugant on SPA + tetracycline medium, were isolated and tested for resistance to albicidin. No growth inhibition was observed when these clones were used as tester strains in an albicidin production assay performed with *X. albilineans* Xa23R1. These results showed that cross-resistance occurs between the albicidin-like product of *X. axonopodis* pv. *vesicatoria* and albicidin produced by *X. albilineans*, suggesting that both molecules are similar. Comparison of chemical characteristics of the two molecules will, however, be necessary to confirm that the two molecules are identical.

[00160] The invention includes the isolation and sequencing of a region of 55,839 bp from *X. albilineans* strain Xa23R1 containing the major gene cluster XALB1 involved in albicidin production. Analysis of this region allowed us to predict the genetic organization of the gene cluster XALB1 which contains 20 ORFs grouped in four or five operons (Figure 1). Because *albXVIII* is a truncated gene, XALB1 genes may be organized in five operons. Therefore, we will from now on consider *albXVII* as part of operon 4 and *albXIX* and *albXX* as part of operon 5. Similar operon-type organizations for antibiotic biosynthesis clusters are well known and have been postulated to facilitate cotranslation of genes within the operon to yield equimolar amounts of proteins for optimal interactions to form the biosynthesis complexes (Cane, 1997). Overlapping genes involved in the same process are also quite common in bacteria (Normark *et al.*, 1983).

[00161] Previous results of transposon mutagenesis and complementation studies (Rott *et al.*, 1996; Rott, unpublished results) are in accordance with the predicted genetic organization of XALB1 described in this study, and allowed us to establish that operons 1, 2 and 3 are involved in albicidin biosynthesis: (i) Tox⁻ mutants with a Tn5-*gusA* insertion site located in DNA fragments B, C, G and D were complemented by cosmid pALB571 and not by cosmid pALB540, confirming that cosmid pALB571 potentially contains the entire operon 1; (ii) Tox⁻ mutants with a Tn5-*gusA* insertion site located in DNA fragments A and H were complemented

by both cosmids pALB540 and pALB571, confirming that both cosmids potentially contain the entire operon 2; (iii) mutant XaAM1 with a Tn5-*gusA* insertion site located in DNA fragment J is the only Tn5 Tox⁻ mutant complemented by cosmid pALB540 and not by cosmid pALB571, confirming that cosmid pALB540 potentially contains the entire operon 3. Our mutagenesis studies did not confirm that operons 4 and 5 are required for biosynthesis of albicidin. The para-aminobenzoate (PABA) is required for the growth of many bacteria probably including *X. albilineans*, suggesting that a mutation in *albXVII* may be lethal and explaining why we did not obtain any mutant disrupted in this gene.

[00162] Putative bidirectional promoters were identified between operons 1 and 2 (Huang et al., 2001) and between 3 and 4 (Figure 7), confirming the prediction of genetic organization of XALB1. The region upstream from operon 1 is 100 % identical to the region upstream from the *xabB* start codon which was described as a functional promoter during the phase of albicidin accumulation in Australian strain Xa13 of *X. albilineans* (Huang et al., 2001). Involvement of several operons in albicidin biosynthesis suppose that they are transcribed during the same time. Promoter activities of regions upstream from putative operons 2, 3, 4 and 5 need to be determined to precise if these promoters are functional during the same growth phase of *X. albilineans* as the promoter upstream from operon 1.

[00163] Potential rho-independent transcription terminators were identified downstream from operons 1, 3 and 4 (Figure 6) confirming prediction of the genetic organization of these three operons. Because operons 2 and 5 are convergent (Figure 1) and separated by a very short region of 22 bp between *albIX* and *albXX*, stop codons may allow transcription termination in the absence of sequences corresponding to potential rho-independent transcription terminators downstream from these operons. It is quite possible that simultaneous transcription of operons 2 and 5 involving the presence of two RNA polymerases (one on each strand of DNA) may cause RNA polymerases to pause at the end of each operon because of steric interference between both polymerase complexes in the same short region.

[00164] The presence of putative RBSs upstream of the ATG start codons of all ORFs, except for *albXVIII*, suggests that these ORFs are translated in *X. albilineans*. The absence of a canonical RBS upstream from *albXVIII* further indicates that this ORF is probably not expressed. GTG and TTG codons (usually valine and leucine codons) generally serve as procaryotic start codons when located near the 5' end of an RNA message, but GTG start codons were also described far from the 5' end of messenger RNA in the bacitracin biosynthesis cluster of *B. licheniformis* (Genbank Accession No. AF184956) or in the bleomycin biosynthetic gene cluster of *S. verticillus* (Genbank Accession No. AF210249). This is in accordance with the fact that the two potential TTG start codons are the first start codons in operons 1 and 4 of XALB1, and that the two potential GTG start codons initiate internal cistrons. The *albI* and *albXVII* genes,

like *xabB* (Huang *et al.*, 2001), use TTG as a start codon, which may impose post-transcriptional control of the rate of gene product formation (McCarthy and Gualerzi, 1990).

[00165] The predicted genetic organization of operons 1 and 2 presents similarities with the organization of the region involved in albicidin production in strain Xa13 of *X. albilineans* from Australia (Huang *et al.* 2000a, Huang *et al.*, 2001). This latter region also contains two divergent operons involved in albicidin production, one comprising the *xabB* gene (similar to *albI*, but with a large deletion) and the *xabC* gene (100% identical to *albII*) and the other containing *thp* gene (100% identical to *albV*). In addition, the sequence between the two operons in strain Xa13 is 100% identical to the sequence between operons 1 and 2, indicating that both clusters are controlled by the same bidirectional promoter. However, transposon mutagenesis studies of Xa13 showed no evidence of another cistron downstream of *xabC* that may be involved in albicidin production (Huang *et al.*, 2000a), suggesting that the Xa13 *xab* operon differs from the Xa23R1 operon 1, which contains two additional genes downstream from *albII* that are potentially involved in albicidin production (*albIII* and *albIV*; refer Figure 1).

[00166] Homology analysis revealed that four NRPS and/or PKS genes are present in XALB1 (Figure 2), and these genes may be involved in the biosynthesis of the albicidin polyketide-polypeptide backbone (*albI*, *albIV*, *albVII* and *albIX*). NRPS and PKS enzymes are generally organized into repeated functional units known as modules, each of which is responsible for a discrete stage of polyketide or polypeptide chain elongation (Cane and Walsh, 1999). Each PKS or NRPS module is made up of a set of three core domains, two of which are catalytic and one of which acts as a carrier, and together are responsible for the central chain-building reactions of polyketide or polypeptide biosynthesis. Both PKS and NRPS core domains utilize analogous acyl-chain elongation strategies in which the growing chain, tethered as an acyl-S-enzyme to the flexible 20 Å long phosphopantetheinyl arm of an acyl carrier protein (ACP) or peptidyl carrier protein (PCP) domain, acts as the electrophilic partner that undergoes attack by a nucleophilic chain-elongation unit, a malonyl- or aminoacyl-S-enzyme derivative, respectively, itself covalently bound to a downstream ACP/PCP domain. In the case of a PKS, the fundamental chain-elongation reaction, a C-C bond-forming step, is mediated by a ketosynthase (KS) domain that catalyzes the transfer of the polyketide acyl chain to an active-site cysteine of the KS domain, followed by condensation with the methylmalonyl- or malonyl-S-ACP by a decarboxylative acylation of the malonyl donor unit. An additional essential component of the core PKS chain-elongation apparatus is an associated acetyltransferase (AT) domain, which catalyzes the priming of the donor ACP sidearm with the appropriate monomer substrate, usually methylmalonyl- or malonyl-CoA. The comparable core domains of an NRPS biosynthetic module function in a chemically distinct but architecturally and mechanistically analogous fashion. In the latter case, the key chain-building reaction, a C-N bond-forming reaction, involves the generation of the characteristic peptide bond by nucleophilic attack of the amino group of an amino acyl-S-PCP

donor on the acyl group of an upstream electrophilic acyl- or peptidyl acyl-S-PCP chain, catalyzed by a condensation (C) domain. In functional analogy to the PKS AT domain, the core of the NRPS module utilizes an adenylation (A) domain to activate the donor amino-acid monomer as an acyl-AMP intermediate, which is then loaded onto the downstream PCP side chain. Both the AT and A domains of the respective PKS and NRPS modules act as important gatekeepers for polyketide or polypeptide biosynthesis, exhibiting strict or at least high specificity for their cognate malonyl-CoA, methylmalonyl-CoA or amino acid substrates. In addition to the basic subset of core domains, each PKS or NRPS also has a special set of dedicated domains responsible both for the initiation of acyl-chain assembly by loading of a starter unit onto the first, furthest upstream PKS/NRPS module, as well as a chain-terminating thioesterase (TE) domain, most often found fused to the last module, that is responsible for detachment of the most downstream covalent acyl enzyme intermediate and off-loading of the mature polyketide or polypeptide chain (Cane and Walsh, 1999).

[00167] XALB1 potentially encodes four PKS modules and seven NRPS modules. Most of the bacterial NRPS gene clusters described up to now are organized in operon-type structures, encoding multi modular NRPS proteins with individual modules organized along the chromosome in a linear order that parallels the order of amino acids in the resultant peptide, following the “colinearity rule” for the NRPS-template assembly of peptides from amino acids (Cane, 1997; Cane *et al.*, 1998; Cane and Walsh, 1999; von Döhren *et al.*, 1999). PKS and NRPS modules are apparently not organized according to this “colinearity rule” for albicidin biosynthesis because of the following features : (i) NRPS and PKS genes are expressed in two divergent operons; (ii) no AT domain was identified in PKS-2 and PKS-3 domains, suggesting involvement of a separate enzyme ; (iii) the A domain of NRPS-2 is not functional, suggesting the involvement of a *trans*-acting A domain ; (iv) a single chain-terminating TE domain was identified in XALB1 which may be responsible of the release of the full length albicidin polyketide-polypeptide backbone from the enzyme complexes. Exception to the “colinearity rule” has also been shown for the syringomycin synthetase of *P. syringae* (Guenzi *et al.*, 1998), for the exochelin synthetase of *Mycobacterium smegmatis* (Yu *et al.*, 1998) and for the bleomycin synthetases of *S. verticillus* (Du *et al.*, 2000).

[00168] On the basis of the deduced functions of individual NRPS and PKS domains we have aligned the four PKS and the seven NRPS modules to suggest two different putative linear models for the synthesis of the albicidin polyketide-peptide backbone (Figure 9). In the Figure, NRPS and PKS domains are abbreviated as follows: A, adenylation; ACP, acyl carrier protein; AL, acyl-CoA ligase; AT, acyltransferase; C, condensation; HBCL, hydroxybenzoate-CoA ligase; KR, ketoreductase; KS, ketoacyl synthase; PCP, peptidyl carrier protein. Asn designates asparagine. X1 and X2 indicate substrates incorporated by NRPS - 1 and 3 and by NRPS-6 and 7, respectively. The crossed A domain in NRPS-2 indicates that this

deleted domain may be not functional. In model 1, (Figure 9A), (i) the PKS-1 module alone is responsible for the initiation of the acyl-chain assembly, (ii) PKS-4 (HBCL) interacts with PKS-2 and PKS-3 as an AT domain to allow acyl transfer and (iii) NRPS-5 interacts with only NRPS-2. In model 2 (Figure 9B) two different modules, PKS-1 and PKS-4, are responsible for this initiation step. Model 2 leads to the biosynthesis of four different polyketide-polypeptide backbones; in this model (i) PKS-1 (AL) and PKS-4 (HBCL) are in competition for initiation of albicidin precursors; (ii) a separate AT enzyme (potentially AlbXIII) interacts with PKS-2 and PKS-3 to allow acyl transfer; (iii) NRPS-5 interacts with NRPS-2; and (iv) NRPS-5 and NRPS-6 are in competition for interaction with NRPS-4.

[00169] Both models are based on the fact that PKS-1 contains the AL and ACP1 domains, and PKS-4 shows homology with the hydroxybenzoate-CoA ligases. In other PKS systems, an N-terminal AL domain is involved in the activation and incorporation of an 3,4-dihydroxycyclo hexane carboxylic acid, a 3-amino-5-hydroxybenzoic acid or a long-chain fatty acid as a starter (Aparicio *et al.*, 1996; Motamedi and Shafiee, 1998; Tang *et al.*, 1998; Duitman *et al.*, 1999). PKS-4 may be also involved in the activation and incorporation of hydroxy-benzoate but this latter domain lacks any ACP or PCP domain, suggesting that PKS-4 is responsible for initiation of the acyl-chain assembly (Figure 9B) onto one of the three ACP domains of AlbI (ACP1, ACP2 or ACP3). The 277 amino-acids preceding the PKS-4 module in AlbVII may be necessary for the intercommunication between AlbVII and AlbI. The presence of two different PKS modules potentially involved in the initiation of the acyl-chain assembly suggests a competition of these two modules for the initiation of two different albicidin polyketide-polypeptide backbones, and this could contribute to the production of multiple, structurally related albicidins by the same cluster XALB1. Production of two different components, one initiated by PKS-4 containing an additional aromatic ring due to incorporation of hydroxybenzoate, may explain why partial characterization of albicidin indicated the presence of a variable number (three or four) of aromatic rings (Huang *et al.*, 2001).

[00170] In AlbI, PKS-1 is followed by the PKS-2 module which contains a KS domain and a KR domain upstream from two ACP domains (ACP2 and ACP3) and it lacks any discernable AT domain. Tandem ACP domains are unusual within PKS modules but have been shown to occur in the biosynthesis of several fungal and bacterial polyketide synthases (Mayorga and Timberlake, 1992; Yu and Leonard, 1995; Takano *et al.*, 1995; Albertini *et al.*, 1995). However, the significance of the tandem ACP domains in these systems has not been solved yet. In our model 2, one of the tandem ACP (ACP2 or ACP3) may interact with PKS-4 for the initiation of an acyl-chain assembly (Figure 9B). The absence of an AT domain in the PKS-2 module suggests that a separate AT domain is indispensable for the elongation of the acyl-chain initiated by this module. Separate AT enzymes encoded elsewhere in the genome were described in other systems for two PKS modules lacking AT domains: malonyl-CoA transacyclase gene

(*fenF*) located immediately upstream from the *B. subtilis* PKS-NRPS *mycA* gene (Duitman *et al.*, 1999) and an AT gene located 20kb upstream from the *M. xanthus* NRPS-PKS *tal* gene (Paitan *et al.*, 1999). We have not identified an AT gene in the gene cluster XALB1 and in the two other genomic regions involved in albicidin production, XALB2 and XALB3, suggesting that the *trans*-acting AT gene may be encoded elsewhere in the genome. However, AlbXIII, which contains the motif GHSxG conserved in AT domains, may be potentially involved in the acyl transfer, but the similarity of AlbXIII with AT domains is not high enough to confirm this potential function of AlbXIII (Figure 10). Figure 10A describes alignment of the conserved motifs in AT domains from RifA-1, -2, -3, RifB-1, RifE-1 (Rifamycin PKSs, August *et al.*, 1998) and BlmVIII (Bleomycin PKS; Du *et al.*, 2000), identical amino acids are shown in bold. Figure 10B describes alignment of AlbXIII (SEQ ID NO. 38), FenF (a malonyl-CoA transacylase located upstream from *mycA*, Duitman *et al.*, 1999) and LipA (a lipase; Valdez *et al.*, 1999); amino acids identical to conserved AT domains motifs are shown in bold.

[00171] AlbXIII contains only four of the eleven amino acids conserved in AT domains of rifamycin PKSs (August *et al.*, 1998) and Bleomycin PKS (Du *et al.*, 2000), and the AlbXIII sequence appears to be more closely related to lipases such as LipA (Valdez *et al.*, 1999) rather than to AT domains (Figure 10). However, FenF, the *trans*-acting AT domain involved in mycosubtilin biosynthesis, contains only seven of the eleven amino acids conserved in AT domains (Duitman *et al.*, 1999; Figure 10). AlbVII, that contains a HBCL domain, may be another candidate for the acyl transfer in PKS-2 (Figure 9A) because HBCL exhibits some similarity with A domains at the level of cores A1, A2, A3, A4, A5 and A6 (Table 6). However, no HBCL involved in such a function has been described in the PKSs characterized so far.

[00172] In AlbI, PKS-2 is followed by the PKS-3 module which contains the KS2 and the PCP1 domains and it lacks any discernable AT or A domain. PKS-3 is located upstream from the NRPS modules and should therefore be involved in the linkage of polyketide and polypeptide moieties. The presence of a PCP domain in the PKS-3 module suggests the involvement of a *trans*-acting A domain rather than an AT domain. A putative candidate for this *trans*-acting A domain is the AlbIV NRPS-5 A domain because of the lack of a C domain in the AlbIV NRPS-5 module. However, by analogy with the BlmVIII PKS module, which is involved in the linkage of polypeptide and polyketide moieties of bleomycin and which contains an AT domain followed by a PCP domain (Du *et al.*, 2000), the presence of a PCP is not incompatible with a possible interaction of the AlbI PKS-3 module with a separate AT domain. This latter *trans*-acting AT domain may be the same that interacts with the AlbI PKS-2 module, the AlbVII PKS-4 module, AlbXIII or an unidentified separate AT domain.

[00173] In AlbI, the PKS-3 module is followed by four NRPS modules. The NRPS-1, 2 and 3 modules display the ordered C, A and PCP domains, suggesting that they are

involved in the incorporation of three amino acid residues. The A domain of the NRPS-2 module exhibits poor consensus at A2, A3, A5, A7, A8 A9 and A10 motifs and lacks completely the A6 motif (Table 6). In addition the NRPS-2 substrate binding pocket is partially deleted (Figure 5). These features strongly suggest that the NRPS-2 A domain is inactive and that the loading of an amino-acid on the NRPS-2 PCP domain (PCP3) is possibly catalyzed by a *trans*-acting A domain as in HMWP1 (Gehring *et al.*, 1998) and BlmIII (Du *et al.*, 2000). A putative candidate for this *trans*-acting A domain is the NRPS-5 A domain present in AlbIV because of the lack of a C domain in NRPS-5 (Figure 2). The additional sequence of 300 amino-acids present in the A domain of NRPS-5 may be necessary for the intercommunication between AlbI and AlbIV. As a consequence of the interaction between NRPS-2 and NRPS-5, a competition between PCP-3 and PCP-5 domains must occur to bind the amino acid activated by the NRPS-5 A domain. A similar competition between two PCP domains was described for syringomycin biosynthesis, during the interaction between SyrB, which contains A and PCP domains, and the last module of SyrE which contains C and PCP domains (Guenzi *et al.*, 1998). The NRPS-4 module contains only a C domain which may transfer the intermediate products synthesized by AlbI to a PCP domain present in an other albicidin synthase. Similar transfers were described for mycosubtilin biosynthesis in which the MycA and MycB C-terminal C domains interact with the MycB and MycC N-terminal A domains, respectively (Duitman *et al.*, 1999). Two different PCP domains may be involved in the transfer of the intermediate products synthesized by AlbI: the PCP-5 and PCP-6 domains which are present in the AlbIV NRPS-5 and AlbIX NRPS-6 modules, respectively. This possible competition between the two NRPS modules that contain two different A domains could also contribute to the production of multiple, structurally related albicidins by the gene cluster XALB1 (Figure 9B). Because of the absence of a C-domain in the AlbIX NRPS-6 module, the intermediate product bound on the AlbIV PCP-5 domain would be necessarily transferred to the AlbIX PCP-7 domain, like the intermediate product bound on AlbIX PCP-6. AlbIX NRPS-7, which contains the single chain-terminating TE domain, may then be responsible for detachment of the mature albicidin polyketide-polypeptide backbone from the complex of enzymes.

[00174] The linear model 1 implies that operon 1 and operon 2 in *X. albilineans* strain Xa23R1 from Florida potentially produce only one albicidin polyketide-polypeptide backbone, with a competition at the level of ACP2/ACP3 and PCP3 and PCP5 which could explain the production by *X. albilineans* of compounds structurally related to albicidin (Figure 9A). The linear model 2 implies that operon 1 and operon 2 in *X. albilineans* strain Xa23R1 from Florida potentially produce four different albicidin polyketide-polypeptide backbones (Figure 9B) because of (i) the competition of AL and HBCL domains for initiation of acyl chain assembly and (ii) the competition of AlbIV NRPS-5 and AlbIX NRPS-6 modules for the incorporation of the next to last amino acid of the albicidin backbone. These four albicidin backbones may lead to the

production of four components structurally very different. The polyketide moieties of the acyl chains initiated by the AlbI AL domain or by the AlbVII HBCL domain may be very different. The polyketide moiety of acyl chains initiated by the AlbVII HBCL domain may be shorter and may contain an additional aromatic ring. The presence of four structurally different metabolites may explain the difficulty observed by Birch and Patil (1985a) to purify albicidin and to determine its chemical structure.

[00175] Homology analysis also revealed that AlbI NRPS-1 and 3 and AlbIX NRPS-6 and 7 specify unusual substrates which seem to contain an amino group and a carboxylate group but to be different from α -amino acids and β -alanine. Identification of several aromatic rings in albicidin (Huang *et al.*, 2001) suggested that NRPS-1, -3, -6 and -7 are involved in incorporation of aromatic substrates. By analogy with the Asp235Val alteration in the β -Ala specificity-conferring code (Du *et al.* 2000), the Asp235Ala alteration in the NRPS-1, -3, -6 and -7 signatures could be consistent with a large distance between the amino group and the carboxylate group in the substrate specified by these modules. Based on this hypothesis, we suggest that operons 3, 4 and 5 are involved in the biosynthesis of two aromatic substrates: the para-aminobenzoate potentially synthesized by AlbXVII (para-aminobenzoate synthase), and the carbamoyl benzoate potentially synthesized by AlbXX (hydroxybenzoate synthase) and AlbXV (carbamoyl transferase). Incorporation of these nonproteinogenic substrates may explain why albicidin is insensitive to proteases (Birch and Patil, 1985a).

[00176] According to biosynthesis model 1 leading to the biosynthesis of only one polyketide-polypeptide albicidin backbone that may correspond to the major component produced by XAlb1, we propose a model allowing prediction of the composition and the structure of albicidin (Figure 11). In the Figure, NRPS and PKS domains are abbreviated as follows: A, adenylation; ACP, acyl carrier protein; AL, acyl-CoA ligase; C, condensation; KR, β -ketoreductase; KS, β -ketoacyl synthase; PCP, peptidyl carrier protein. C atoms of albicidin backbone are numbered 1 to 38. Bold methyl groups correspond to methylation of the albicidin backbone by AlbII or AlbVI. In this model, albicidin biosynthesis is initiated by loading of an acetyl-CoA by PKS-1 (step 1), and the chain product is elongated by incorporation of (i) malonyl-CoA by PKS-2 and PKS-3 (steps 2 and 3), (ii) para-aminobenzoate or carbamoyl benzoate by NRPS-1 and NRPS-3 (steps 4 and 6), (iii) asparagine by NRPS-2 coupled to NRPS-5 (step 5) and (iv) para-aminobenzoate or carbamoyl benzoate by NRPS-6 and NRPS-7 (steps 7 and 8). The presence of the KR domain in the PKS-2 module may lead to the formation of an hydroxyl group at the C₂ atom of the albicidin backbone. This hydroxyl group might be methylated by AlbVI (O-methyltransferase). The acyl chain may also be modified by AlbII (C-methyltransferase) at C₁₃ or C₁₄.

[00177] The chemical composition ($C_{40}O_{15}N_6H_{35}$), the molecular weight (839), and the structure of the putative XALB1 product are in accordance with the partial characterization of albicidin published by Birch and Patil (1985a) which indicated that albicidin contains approximately 38 carbon atoms and a carboxylate group and that the molecular weight of albicidin was about 842. The presence of two ester linkages in our predicted albicidin structure is also in accordance with the fact that albicidin is detoxified by the AlbD esterase (Zhang and Birch, 1997). However, an unpublished albicidin analysis cited by Huang *et al.* (2001) indicated the presence of (i) two OCH₃ groups and not one as in our predictive albicidin structure, (ii) one CN linkage and not eleven as in our predictive albicidin structure and (iii) a trisubstituted double bond that is not present in the putative XALB1 product.

[00178] In conclusion, homology analysis of XALB1 revealed unprecedented features for hybrid polyketide-peptide biosynthesis in bacteria involving a *trans*-action of four PKS and seven NRPS separate modules which could contribute to the production of multiple, structurally related polyketide-peptide compounds by the same gene cluster. Characterization of the full chemical structure of albicidin may be necessary to validate these models. Four NRPS modules seem to activate a very unusual substrate. Over- expression and purification of A domains from these four NRPS modules will be necessary to examine their substrate specificities. Substrate specificity of each A domain will therefore be determined by analysis of the ATP-PPi exchange reaction with different substrate putatively incorporated into albicidin. Investigating albicidin backbone biosynthesis will be of great interest because such information adds to the limited knowledge as to how PKS and NRPS interact and how they might be manipulated to engineer novel molecules, and may explain how *X. albilineans* produces several structurally related, toxic compounds.

[00179] Cloning and sequencing of XALB2 showed that the same phosphopantetheinyl transferase is required for albicidin production in an *X. albilineans* strain from Florida and in an *X. albilineans* strain from Australia (Huang *et al.*, 2000b), explaining the precedented results showing that strain LS156 mutated in *xabA* (100% identical to *albXXI*) was not complemented by pALB540, pALB571 and pALB639 (Rott *et al.*, 1996). Mutant LS156 was shown to be complemented by a construction containing the coding sequence of *xabA* in fusion with *lacZ*, revealing that *xabA* is required for albicidin production and that no other cistron downstream from *xabA* was involved in albicidin production (Huang *et al.*, 2000b). However, this complementation study did not allow determination of whether *xabA* is transcribed as a part of a larger operon. Here we disclose the complementation of mutant AM37 with a 2986 bp insert from *X. albilineans* containing *albXXI* (100% identical to *xabA*), confirming that *albXXI* is involved in albicidin biosynthesis and indicating that the promoter of *albXXI* is present in the 2986 bp insert and that *albXXI* is not expressed as part of a operon.

[00180] Cloning and sequencing of XALB3 showed that a heat shock protein HtpG was involved in albicidin production in *X. albilineans*. The heat shock protein HtpG is an *Escherichia coli* homologue of eukaryotic HSP90 molecular chaperone. Hsp90 from eukaryotes has been demonstrated to possess chaperone activity (Jakob *et al.*, 1995), acting as a non-ATP dependent "holder," and it also has an important role in signal transduction and the cell cycle. This protein is essential in both drosophila and yeast (Borkovich *et al.*, 1989; Cutforth and Rubin, 1994). In contrast, the HtpG gene can be deleted in *E. coli* with no effect on the viability of the strain with the exception of decreased growth rate at high temperatures (Bardwell and Craig, 1988). The *in vivo* role of the HtpG protein remains unknown. However, preliminary results indicated that HtpG facilitates *de novo* protein folding in stressed *E. coli* cells, presumably by expanding the ability of the DnaK-DnaJ-GrpE molecular chaperone system to interact with newly synthesized polypeptides (Thomas and Baneyx, 2000). Furthermore, HtpG was copurified in *E. coli* with MccB17 synthetase, an enzyme involved in the biosynthesis of the peptide antibiotic microcin B17 which inhibits DNA replication by induction of the SOS repair system, suggesting the requirement of HtpG for production of the antibiotic (Li *et al.*, 1996). However, when microcin B17 production by the *E. coli* strain deleted for HtpG was compared to the one of the parental strain, there was no effect on microcin B17 production *in vivo*. This result implied that the copurification of HtpG with the MccB17 synthetase was potentially an artifact, or that another *E. coli* chaperone could substitute for HtpG (Milne *et al.*, 1999). To examine the effect of HtpG on the reconstitution of MccB17 synthetase *in vitro*, the chaperone was expressed and purified as a fusion to a hexahistidine (His₆) tag. Addition of the His₆-HtpG did not stimulate MccB17 synthetase reconstitution or heterocyclisation activity *in vitro*, suggesting that HtpG mediates complex assembly or stabilizes protein subunits prior to the hetero-oligomerisation (Milne *et al.*, 1999). Based on these results, we suggest that the function of AlbXXII is to mediate complex assembly by facilitating *de novo* protein folding of PKS and NRPS enzymes (AlbI, AlbIV, AlbVII and AlbIX) involved in the albicidin backbone biosynthesis.

[00181] Characterization of the complete sequence of XALB1, XALB2 and XALB3 clusters enables one to characterize all enzymes of the albicidin biosynthesis pathway including structural, resistance, secretory and regulatory elements, and to engineer overproduction of albicidin. For example one may insert expression enhancing DNA into the genome of *X. albilineans* in a position operable to enhance expression of the Albicidins Biosynthesis Gene Clusters. One may also modify naturally occurring Albicidins to obtain additional non-naturally occurring antibiotics by adding DNA encoding additional enzymes selected to produce a modified albicidin like molecule. This approach will allow (i) the purification of albicidin and the other compounds structurally related and potentially produced by the same biosynthesis apparatus; (ii) the characterization of chemical structure of albicidin; (iii) the investigation of mode of action of

albicidin in the pathogenesis of *X. albilineans* in sugarcane; and (iv) the characterization of the bactericidal activity of albicidin. For example one may also increase the resistance of plants to damage from *X. albilineans* infection by inserting one or more of the resistance genes identified herein into the genome of the plant. One may also provide materials to prevent damage by albicidin produced by *X. albilineans* by applying an agent that blocks expression of the Albicidin Biosynthesis Gene Clusters to the plant to be protected. One may also use portions of the DNA of the Albicidin Biosynthesis Gene Clusters to obtain agents useful in blocking expression of albicidin by screening materials against a modified host cell line that expresses the Albicidin Biosynthesis Gene Clusters and selecting for materials that stop or decrease albicidin production.

Table 1 : Bacterial strains and plasmids used in this study		
	Relevant characteristics ^a	Reference or source
Strains		
<i>E. coli</i>		
DH5 α	F-/80dlacZ Δ M15 Δ (lacZYA-argF)U169 <i>deoR recA1 endA1 hsdR17</i> (r _k ⁻ m _k ⁺) supE44 thi-1 <i>gyrA96 relA1</i>	Gibco-BRL
DH5 α MCR	DH5 α <i>mcrA mcrBC mrr</i>	"
Xcv 91-11B	Wild type strain of <i>Xanthomonas axonopodis</i> pv. <i>vesicatoria</i> from tomato (race 3)	Astua-Monge <i>et al.</i> , 2000
Xcv 91-11BR1	Spontaneous Rif ^r derivative of Xcv 91-11B	This study
DH5 α KT	<i>Escherichia coli</i> DH5 α strain transformed by both pUFR043 and pLAFR3 plasmids	"
DH5 α Alb ^r	Spontaneous Alb ^r derivative of DH5 α	"
DH5 α Alb ^r KT	DH5 α Alb ^r transformed by both pUFR043 and pLAFR3 plasmids	"
Plasmids		
PBR325	Tc ^r , Ap ^r , Cm ^r	Gibco-BRL
pBCKS (+)	Cm ^r	Stratagene
pBluescript II KS (+)	Ap ^r	"
PRK2073	PRK2013 derivative, Km ^s (<i>npt::Tn7</i>), Sp ^r , Tra ⁺ , helper plasmid	Leong <i>et al.</i> , 1982
pUFR043	IncW Mob ⁺ LacZ α Gm ^r , Km ^r , Cos	De Feyter and Gabriel, 1991
pAlb540	47 kb insert from Xa23R1 in pUFR043, Gm ^r , Km ^r	Rott <i>et al.</i> , 1996
pAlb571	36.8 kb insert from Xa23R1 in pUFR043, Gm ^r , Km ^r	"
pAlb639	36 kb insert from Xa23R1 in pUFR043, Gm ^r , Km ^r	"
pAM15.1	24 kb <i>EcoR</i> I fragment carrying Tn5 and flanking sequences of mutant AM15 in pBR325, Km ^r , Tc ^r , Ap ^r , Cm ^r	"
pAM40.2	11 kb <i>EcoR</i> I fragment carrying Tn5 and flanking sequences of mutant AM40 in pBR325, Km ^r , Tc ^r , Ap ^r , Cm ^r	"
pAM45.1	12 kb <i>EcoR</i> I fragment carrying Tn5 and flanking sequences of mutant AM45 in pBR325, Km ^r , Tc ^r , Ap ^r , Cm ^r	"
pAM12.1	13 kb <i>EcoR</i> I fragment carrying Tn5 and flanking sequences of mutant AM12 in pBR325, Km ^r , Tc ^r , Ap ^r , Cm ^r	A
pAM36.2	9 kb <i>EcoR</i> I fragment carrying Tn5 and flanking sequences of mutant AM36 in pBR325, Km ^r , Tc ^r , Ap ^r , Cm ^r	A
pAlb389	37 kb insert from Xa23R1 in pUFR043, Gm ^r , Km ^r	This study
pAC389.1	2.9 kb insert from Xa23R1 in pUFR043, Gm ^r , Km ^r	"
pAlb639A	9.4 kb insert from Xa23R1 in pUFR043, Gm ^r , Km ^r	"
PEV639	2.6 kb <i>Sal</i> I insert from Xa23R1 in pUFR043, Gm ^r , Km ^r	"
pBC/A'	7.5 kb <i>Kpn</i> I fragment carrying a part of fragment A from pAlb571 in pBCKS (+), Cm ^r	"
pBC/AF	15.2 kb <i>EcoR</i> I fragment carrying fragments A and F from pALB540 in pBCKS (+), Cm ^r	"
pBC/B	11.0 kb <i>Kpn</i> I fragment B from pAlb571 in pBCKS (+), Cm ^r	"
pBC/C	6.0 kb <i>Kpn</i> I fragment C from pAlb571 in pBCKS (+), Cm ^r	"
pBC/E	2.8 kb <i>Kpn</i> I fragment E from pAlb571 in pBCKS (+), Cm ^r	"
pBC/F	2.5 kb <i>Kpn</i> I- <i>EcoR</i> I fragment F from pAlb571 in pBCKS (+), Cm ^r	"
pBC/G	1.9 kb <i>EcoR</i> I fragment G from pAlb571 in pBCKS (+), Cm ^r	"

Table 1 : Bacterial strains and plasmids used in this study		
	Relevant characteristics ^a	Reference or source
pBC/I	1.4 kb <i>Kpn</i> I- <i>Eco</i> R I fragment I from pAlb571 in pBCKS (+), Cm ^r	"
pBC/J	0.6 kb <i>Eco</i> R I fragment J from pALB540 in pBCKS (+), Cm ^r	"
pBC/K	4.7 kb <i>Eco</i> R I fragment K from pALB540 in pBCKS (+), Cm ^r	"
pBC/L	0.4 kb <i>Eco</i> R I fragment L from pALB540 in pBCKS (+), Cm ^r	"
pBC/N	7.7 kb <i>Eco</i> R I fragment N from pALB540 in pBCKS (+), Cm ^r	"
pUFR043/D=	2.2 kb <i>Eco</i> R IBSau3A I fragment carrying a part of fragment D from pAlb571 in pUFR043	"
pAM1	5 kb <i>Eco</i> R I fragment carrying Tn5 and flanking sequences of mutant AM1 in pBluescript II KS (+), Km ^r , Ap ^r	"
pAM4	12 kb <i>Eco</i> R I fragment carrying Tn5 and flanking sequences of mutant AM4 in pBluescript II KS (+), Km ^r , Ap ^r	"
pAM7	6 kb <i>Eco</i> R I fragment carrying Tn5 and flanking sequences of mutant AM7 in pBluescript II KS (+), Km ^r , Ap ^r	"
pAM10	7 kb <i>Eco</i> R I fragment carrying Tn5 and flanking sequences of mutant AM10 in pBluescript II KS (+), Km ^r , Ap ^r	"
pAM29	10 kb <i>Eco</i> R I fragment carrying Tn5 and flanking sequences of mutant AM29 in pBluescript II KS (+), Km ^r , Ap ^r	"
pAM37	6 kb <i>Eco</i> R I fragment carrying Tn5 and flanking sequences of mutant AM37 in pBR325, Km ^r , Tc ^r , Ap ^r , Cm ^r	"
pAM52	5 kb <i>Eco</i> R I fragment carrying Tn5 and flanking sequences of mutant AM52 in pBluescript II KS (+), Km ^r , Ap ^r	"
PLAFR3	IncP, Mob+, <i>LacZ</i> α, Tc ^r , <i>cos</i>	Staskawicz <i>et al.</i> , 1987
PLAFR3XhoI	pLAFR3 with a <i>Xho</i> I site added to the <i>Bam</i> HI site using an adaptator	This study
pBC/Op4Δ	<i>Bam</i> HI- <i>Pst</i> I fragment from pALB540 cloned between <i>Bam</i> HI and <i>Pst</i> I sites of pBCKS(+)	"
pBC/Op4ΔXhoI	pBC/Op4Δ with a <i>Xho</i> I site created by directed mutagenesis upstream from the <i>Bfr</i> I site	"
pBC/Op4Δ/XALB2	<i>Eco</i> RI DNA fragment from pAC389.1 cloned into the <i>Eco</i> RI site of pBC/Op4ΔXhoI	"
pBC/Op3-4/XALB2	<i>Bfr</i> I DNA fragment from pALB540 cloned into the <i>Bfr</i> I site of pBC/Op4Δ/XALB2	"
pBKS/XALB3	<i>Sal</i> I DNA fragment from pEV639 cloned into the <i>Sal</i> I site of pBluescript II KS (+)	"
pBKS/XALB3XhoI	pBKS/XALB3 with a <i>Xho</i> I site created by directed mutagenesis to substitute the <i>Sal</i> I site located on the <i>Kpn</i> I side of the polylinker	"
pBKS/Op3-4/XALB2-3	<i>Xho</i> I DNA fragment from pBC/Op3-4Δ/XALB2 cloned into the <i>Sal</i> I site of pBKS/XALB3XhoI	"
pOp3-4/XALB2-3	<i>Xho</i> I DNA fragment from pBKS/Op3-4/XALB2-3 cloned into the <i>Xho</i> I site of pLAFR3XhoI	"
pEValbXXII	<i>albXXII</i> in fusion with <i>LacZ</i> in pUFR043, Gm ^r , Km ^r	"
pEVHtpG	<i>E. coli htpG</i> in fusion with <i>LacZ</i> in pUFR043, Gm ^r , Km ^r	"
PGemT	ColE1 replicon, Ap ^r , <i>LacZ</i> α, single 3'-T overhangs at the insertion site	Promega
PGemT/albXXII	PCR fragment containing <i>albXXII</i> cloned into pGemT	This study
PGemT/albXXII bis	<i>Bgl</i> II- <i>Sal</i> IDNA fragment from pBKS/XALB3 cloned between the <i>Bgl</i> II and <i>Sal</i> I sites of pGemT/albXXII	"
PGemT/HtpG	PCR fragment containing the <i>E. coli htpG</i> gene cloned into pGemT	"

Table 1 : Bacterial strains and plasmids used in this study		
	Relevant characteristics ^a	Reference or source
DNA Fragment		
PR37	1.1 kb <i>Hind</i> III- <i>Hind</i> III from pAM37	"

^a Ap^r, Cm^r, Gm^r, Km^r, Rif^r, Sp^r, Tc^r: resistant to ampicillin, chloramphenicol, gentamycin, kanamycin, rifampicin, spectinomycin, tetracycline, respectively. Tox⁻, deficient in albicidin production. Tn5-*gusA*, Tn5-*uidA1* Km^r Tc^r, forms transcriptional fusions. Alb^r, Ap^r, Gm^r, Rif^r and Tc^r: resistant to albicidin, ampicillin, gentamycin, rifampicin and tetracycline, respectively.

Table 2: Analysis of putative translational signals and location of all putative orfs identified in the XALB1 gene cluster

Intergenic spacing between consecutive ORFs in each putative operon	ORF	Potential RBS ^a (distance from start codon)	Start codon (position)	Stop codon (position)
Operon 1 (strand +)				
	<i>albI</i>	GAGGG (5 b)	TTG (30166)	TAG (50805)
45 b	<i>albII</i>	GAGGG (5 b)	ATG (50851)	TAA (51882)
ATG overlaps TAA	<i>albIII</i>	GAGGG (7 b)	ATG (51882)	TGA (52385)
GTG overlaps TGA	<i>albIV</i>	GAGG (7 b)	GTG (52382)	TAA (55207)
Operon 2 (strand -)				
	<i>albV</i>	GGAGG (8 b)	ATG (29929)	TAA (29210)
87 b	<i>albVI</i>	AAGG (4 b)	GTG (29122)	TGA (28262)
61 b	<i>albVII</i>	GAG (4 b)	ATG (28200)	TAG (25903)
7 b	<i>albVIII</i>	AGGTG (4 b)	ATG (25895)	TAA (24903)
20 b	<i>albIX</i>	GGTG (3 b)	ATG (24882)	TGA (19003)
Operon 3 (strand -)				
	<i>albX</i>	GGGGG (8 b)	ATG (14497)	TGA (14246)
81 b	<i>albXI</i>	AGGAAA (6 b)	ATG (14164)	TGA (13217)
5 b	<i>albXII</i>	GGCCTGA (5 b)	ATG (13211)	TAA (11856)
36 b	<i>albXIII</i>	GGGG (3 b)	ATG (11819)	TAA (10866)
12 b	<i>albXIV</i>	GGAG (8 b)	ATG (10853)	TAG (9363)
41 b	<i>albXV</i>	GGAA (6 b)	ATG (9321)	TAG (7567)
208 b	<i>albXVI</i>	GGAGG (4 b)	ATG (7358)	TAG (7092)
Operon 4 (strand +)				
	<i>albXVII</i>	GGGAGG (5 b)	TTG (14909)	TGA (17059)
274 b	<i>albXVIII</i>	GCTCAG (8 b)	ATG (17334)	TGA (17747)
Overlap (17 b)	<i>albXLX</i>	AGG (9 b)	ATG (17728)	TGA (18330)
41 b	<i>albXX</i>	GCAA (8 b)	ATG (18372)	TAG 18980)

^a: Ribosomal Binding Site

Table 3: Deduced functions of the ORFs in the major albicidin biosynthetic cluster X-ALB1			
ORF	Number of amino acids	Sequence homolog ^a	Proposed function ^{b, c}
Operon 1 <i>AlbI</i>	6879	XabB (AAK15074)	Polyketide- peptide synthase <u>PKS modules</u> <u>PKS domains</u> PKS-1 AL ACP1 PKS-2 KS1 KR ACP2 ACP3 PKS-3 KS2 PCP1 <u>NRPS modules</u> <u>NRPS domains</u> NRPS-1 C A PCP2 NRPS-2 C <u>A</u> PCP3 NRPS-3 C A PCP4 NRPS-4 C
<i>Alibi</i>	343	XabC (AAK15075)	C-methyltransferase
<i>AlbIII</i>	167	ComAB (CAA71583)	Activator of <i>alb</i> genes transcription
<i>AlbIV</i>	941	MycA (T44806) WbpG (E83253)	Peptide synthase <u>NRPS module</u> <u>NRPS domains</u> NRPS-5 A PCP5
Operon 2 <i>AlbV</i>	239	Thp (AAK15074)	No function (transposition)
<i>AlbVI</i>	286	TcmP (AAA67510)	O-methyltransferase
<i>AlbVII</i>	765	HbaA (A58538)	4-hydroxybenzoate CoA ligase
<i>albVIII</i>	330	SyrP (AAB63253)	Regulation
<i>AlbIX</i>	1959	DhbF (CAB04779)	Peptide synthase <u>NRPS modules</u> <u>NRPS domains</u> NRPS-6 A PCP6 NRPS-7 C A PCP7
Operon 3 <i>AlbX</i>	83	MbtH (O05821)	Unknown
<i>AlbXI</i>	315	SyrC (U25130)	Thioesterase
<i>AlbXII</i>	451	BoxB (AAK006000.1)	Unknown
<i>albXIII</i>	317	hp ^d (AAK25001)	Esterase
<i>albXIV</i>	496	ActII-2 (p46105)	Albicidin transporter
<i>AlbXV</i>	584	hp ^d (08390)	Carbamoyl transferase
<i>AlbXVI</i>	88	OrfA (AAC03166)	No function (transposition)
Operon 4 <i>albXVII</i>	716	PabAB (CAC22117)	Para-amino benzoate synthase
Operon 5 <i>albXVIII</i>	137	ADCL (AAG06352)	No function (not functional)
<i>albXIX</i>	200	McbG (P05530)	Immunity against albicidin
<i>albXX</i>	202	UbiC (S25660)	4-hydroxybenzoate synthetase

^aProtein accession numbers in Genbank are given in parentheses.

^bNRPS and PKS domains are abbreviated as follows: A, adenylation; ACP, acyl carrier protein; AL, acyl CoA ligase; C, condensation; KR, ketoreductase; KS, ketoacyl synthase; PCP, peptidyl carrier protein.

^cUnderlined domains are likely inactive due to the lack of highly conserved motifs.

^dhypothetical protein

Table 4: Summary of results obtained from BLAST analyses.

Putative Alb protein	No. of aa residues	Protein homolog	Origin	Genbank accession #	Score	Expect	Identities	Positives	Gaps
AlbI	6879								
PKS-1		XabB (4801 aa) SafB (1770 aa)	<i>Xanthomonas albilineans</i> <i>Myxococcus xanthus</i>	AAK15074 AAC44128	1352 bits (3498) 231 bits (589)	0.0 2e-59	730/730 (100%) 175/532 (32%)	730/730 (100%) 269/532 (49%)	- 23/532 (4%)
PKS-2		XabB (4801 aa) PksM (4273 aa)	<i>X. albilineans</i> <i>Bacillus subtilis</i>	AAK15074 CAB13603	3464 bits (8983) 887 bits (2292)	0.0 0.0	1882/1882 (100%) 626/1896 (33%)	1882/1882 (100%) 938/1896 (49%)	- 140/1896=7%
PKS-3		XabB (4801 aa) PksM (4273 aa)	<i>X. albilineans</i> <i>B. subtilis</i>	AAK15074 CAB13603	1274 bits (3296) 577 bits (1486)	0.0 e-163	653/653 (100%) 293/584 (50%)	653/653 (100%) 391/584 (66%)	- 17/584 (2%)
NRPS-1		XabB (4801 aa) NosA (4379 aa)	<i>X. albilineans</i> <i>Nostoc sp.</i>	AAK15074 AF204805	1934 bits (5010) 618 bits (1594)	0.0 e-176	1035/1046 (99%) 398/1104 (36%)	1039/1046 (99%) 586/1104 (53%)	- 86/1104 (7%)
NRPS-2		NosA (4379 aa) Peptide synthase (5060 aa)	<i>Nostoc sp</i> <i>Anabaena sp.</i>	AF204805 CAC01604	416 bits (1069) 402 bits (1034)	e-115 e-111	337/1127 (29%) 315/1073 (29%)	496/1127 (43%) 479/1073 (44%)	128/1127 (11%) 114/1073 (10%)
NRPS-3		XabB (4801 aa) NosA (4379 aa)	<i>X. albilineans</i> <i>Nostoc sp.</i>	AAK15074 AF204805	1847 bits (4784) 610 bits (1573)	0.0 e-173	997/1044 (95%) 392/1069 (36%)	1007/1044 (96%) 571/1069 (52%)	- 86/1069 (8%)
NRPS-4		XabB (4801 aa) NosC (3317 aa)	<i>X. albilineans</i> <i>Nostoc sp.</i>	AAK15074 AAF17280	889 bits (2297) 240 bits (613)	0.0 2e-62	468/468 (100%) 156/438 (35%)	468/468 (100%) 229/438 (51%)	- 20/438 (4%)
AlbII	343	XabC (343 aa) MtmMII (326 aa) TcmO (339 aa)	<i>X. albilineans</i> <i>Streptomyces argillaceus</i> <i>S. glaucescens</i>	AAK15075 AAD55584 P39896	633 bits (1633) 144 bits (361) 81.7 bits (199)	0.0 1e-34 1e-14	343/343 (100%) 98/323 (30%) 79/314 (25%)	343/343 (100%) 154/323 (47%) 140/314 (44%)	- 4/323 (1%) 12/314 (3%)
AlbIII	167	<i>comA</i> operon protein 2 (136 aa) ComAB (116 aa)	<i>E. coli</i> <i>Bacillus licheniformis</i>	AAC74756 CAA71583	133 bits (335) 97.6 bits (242)	1e-30 8e-20	68/135 (50%) 53/111 (47%)	89/135 (65%) 68/111 (60%)	- 1/111 (0%)
AlbIV	941								
PKS-4		BA3 (6359 aa) WbpG (377 aa)	<i>B. licheniformis</i> <i>Pseudomonas aeruginosa</i>	AAC06348 E83253	361 bits (926) 81.6 bits (200)	2e-98 4e-15	190/441 (43%) 44/119 (36%)	267/441 (60%) 70/119 (57%)	14/441 (3%) 4/119 (3%)
AlbV	239	Thp (240 aa) IS transposase (260 aa)	<i>X. albilineans</i> <i>Yersinia pestis</i>	nd AAC82714	nd 160 bits (404)	0.0 1e-38	240/240 (100%) 87/183 (47%)	240/240 (100%) 122/183 (66%)	- 2/183 (1%)
AlbVI	286	Hypothetical protein TcmP (276 aa)	<i>Mycobacterium tuberculosis</i> <i>Pasteurella multocida</i>	AAK46042 AAK03406	138 bits (347) 36.6 bits (83)	6e-32 0.24	92/224 (41%) 32/132 (28%)	125/224 (55%) 65/132 (49%)	18/224 (8%) 29/197 (6%)

Table 4: Summary of results obtained from BLAST analyses.

Putative Alb protein	No. of aa residues	Protein homolog	Origin	Genbank accession #	Score	Expect	Identities	Positives	Gaps
AlbVII	765	4-hydroxybenzoate-CoA ligase (539 aa)	<i>Rhodopseudomonas palustris</i>	AAA62604	203 bits (513)	5e-51	156/492 (31%)	242/492 (48%)	31/492 (6%)
AlbVIII	330	SyrP Like (339 aa) SyrP (353 aa)	<i>S. verticillus</i> <i>Pseudomonas syringae</i>	AF210249 AAB63253	245 bits (619) 182 bits (458)	6e-64 5e-45	130/309 (42%) 106/306 (34%)	182/309 (58%) 155/306 (50%)	2/309 (0%) 4/306 (1%)
AlbIX	1959								
NRPS-6		XabB (4801 aa) DhbF (1278 aa)	<i>X. albilineans</i> <i>B. subtilis</i>	AAK15074 CAB15186	481 bits (1239) 354 bits (908)	e-135 1e-96	286/608 (47%) 222/608 (36%)	374/608 (61%) 341/608 (55%)	23/208 (3%) 21/608 (3%)
NRPS-7		XabB (4801 aa) NosA (4379 aa)	<i>X. albilineans</i> <i>Nostoc</i> sp.	AAK15074 AF204805	874 bits (2258) 551 bits (1420)	0.0 e-155	515/1110 (46%) 388/1148 (33%)	682/1110 (61%) 583/1148 (49%)	52/1110 (4%) 84/1148 (7%)
AlbX	83	Hypothetical protein (72 aa) MbtH (71 aa)	<i>P. aeruginosa</i> <i>M. tuberculosis</i>	AAG05800 CAB08480	75.6 bits (185) 59 bits (142)	1e-13 9e-09	34/61 (55%) 25/55 (45%)	44/61 (71%) 37/55 (66%)	- -
AlbXI	315	SyrC (433 aa) Hydrolase (261 aa)	<i>P. syringae</i> <i>S. coelicolor</i>	AAA85161 CAA16200	34.4 bits (78) 34 bits (77)	1.9 2.9	23/93 (24%) 19/60 (31%)	40/93 (42%) 30/60 (49%)	- -
AlbXII	451	BoxB (473 aa)	<i>Azoarcus evansii</i>	AAK00599	293 bits (751)	3e-78	174/448 (38%)	243/448 (53%)	12/448 (2%)
AlbXIII	317	Hypothetical protein (335 aa) Plasma PAF acetylhydrolase (444 aa)	<i>Caulobacter crescentus</i> <i>Canis familiaris</i>	AAK25001 AAC48484	99.5 bits (247) 37.5 bits (86)	5e-200	88/296 (29%) 43/156	125/296 (41%) 56/156	5/296 (1%) 44/156 (28%)
AlbXIV	496	Putative trans-membrane efflux protein (505 aa) AlbF, putative albicidin efflux pump (496 aa)	<i>S. coelicolor</i> <i>X. albilineans</i>	CAB90983 AF403709	225 bits (574) 736 bits (1900)	0	154/465 (33%) 496/496 (100%)	240/465 (51%) 496/496 (100%)	8/465 (1%) -
AlbXV	584	Probable carbamoyl transferase (585 aa) BlmD (545 aa)	<i>P. aeruginosa</i> <i>S. verticillus</i>	AAG08390 AAG02370	201 bits (513) 192 bits (506)	1e-50 1e-47	158/458 (34%) 149/441 (33%)	222/458 (47%) 209/441 (46%)	39/458 (8%) 33/441 (7%)
AlbXVI	88	Transposase (363 aa) Transposase OrfA (88 aa)	<i>X. axonopodis</i> <i>Desulfovibrio vulgaris</i>	AF263433 AAC03166	64.8 bits (157) 61.0 bits (147)	2e-10 3e-09	27/45 (60%) 29/54 (53%)	40/45 (88%) 38/54 (69%)	- -

Table 4: Summary of results obtained from BLAST analyses.

Putative Alb protein	No. of aa residues	Protein homolog	Origin	Genbank accession #	Score	Expect	Identities	Positives	Gaps
AlbXVII	716	Para-aminobenzoate synthase (723 aa)	<i>Streptomyces griseus</i>	CAC22117	503 bits (1295)	e-141	302/699 (43%)	409/699 (58%)	36/699 (5%)
AlbXVII I	137	4-amino-4-deoxychorismate lyase (271 aa)	<i>P. aeruginosa</i>	AAG06352	81.4 bits (200)	4e-15	46/105 (43%)	65/105 (61%)	-
AlbXIX	200	McbG (187 aa)	<i>E. coli</i>	CAA30724	60.5 bits (145)	9e-09	36/141 (25%)	58/141 (40%)	5/141 (3%)
AlbXX	202	4-hydroxybenzoate synthase (202 aa)	<i>E. coli</i>	AAC77009	45.6 bits (107)	5e-04	42/161 (26%)	21/161 (13%)	-
AlbXXI	278	XabA (278aa)	<i>X. albilineans</i>	AAG28384	430 bits (1106)	0	278/278 (100%)	278/278 (100%)	-
AlbXXII	634	Heat shock protein HtpG (634)	<i>P. aeruginosa</i>	AAG04985	1051 bits (2688)	0	523/634 (82%)	588/634 (92%)	-
		Heat shock protein HtpG (624)	<i>E. coli</i>	AAC73575	743 bits (1899)	0	376/624 (60%)	476/624 (76%)	4/624 (0%)

Table 5 : Comparison of conserved sequences in C domains of peptide synthetases and in putative C domains of the Alb modules

Core	Sequences conserved in peptide synthetases*	Sequence	Alb module
C1	SxAQxR (L/M) (W/Y) xL	TYAQERLWLV STAQERMWFL <i>SYAQERLWLV</i> SLFQERLWFLV SYQQERLWFLV	NRPS-1 NRPS-2 NRPS-3 NRPS-4 NRPS-7
C2	RHExLRTxF	<i>RHEVLRTRF</i> RHAVLRTHF RHEILRTRF RHETLRTRI	NRPS-1 and NRPS-3 NRPS-2 NRPS-4 NRPS-7
C3	MHHxISDG (W/V) S	IHHIISDGWS IHHIVFDGWS MHHLIYDAWS MHHIICDGWS	NRPS-1 and NRPS-3 NRPS-2 NRPS-4 NRPS-7
C4	YxD (F/Y) AVW	YADYALW YADYARW YADYAIW YADYATW	NRPS-1 and NRPS-3 NRPS-2 NRPS-4 NRPS-7
C5	(I/V) Gx FVNT (Q/L) (C/A) xR	<i>IGFFINILPLR</i> IGLFVNTLAVR IGFFVNILAVR	NRPS-1, NRPS-3 and NRPS-4 NRPS-2 NRPS-7
C6	(H/N) QD (Y/V) PFE	HQSVPFEE HQDVPFEE NQALPFEE HRALPFEE	NRPS-1 and NRPS-3 NRPS-2 NRPS-4 NRPS-7
C7	RDxSRNPL	RDSSQIPL RDTARNPL RDTSRIPL RDSSQIPL	NRPS-1 and NRPS-3 NRPS-2 NRPS-4 NRPS-7

*Sourced from Marahiel *et al.*, 1997

Table 6 : Comparison of conserved sequences in A domains of peptide synthetases and in putative A domains of the Alb modules

Core	Sequences conserved in peptide synthetases*	Sequence	Alb module
A1	L (T/S) YxEL	WSYAQL LSYAQL MSYGQL FSYRQL LSYAQL	NRPS-1 and NRPS-3 NRPS-2 NRPS-5 PKS-4 NRPS-6 and NRPS-7
A2	LKAGxAYL (V/L) P (L/I) D	FKAGACYVPID SLCGAASVLID MKAGAAAYVPID LAGGLVFAPIN LKAGGCYVPLD	NRPS-1 and NRPS-3 NRPS-2 NRPS-5 PKS-4 NRPS-6 and NRPS-7
A3	LAYxxYTSG (S/T) TGxPKG	LACVMVTSGSTGRPKG ?TRTIMVESGSLSSRL? PVYCIYTSGSTGSPKG PAVMICTSGSTGTPKA <i>LAYVMYTSGSTGRPKG</i>	NRPS-1 and NRPS-3 NRPS-2 NRPS-5 PKS-4 NRPS-6 et NRPS-7
A4	FDxS	FAVS FDAA FDLT FAYG FAIS	NRPS-1 and NRPS-3 NRPS-2 NRPS-5 PKS-4 NRPS-6 and NRPS-7
A5	NxYGPTE	NNYGCTE ?AAYGNAE? NEYGPTE DGIGCTE YIYGCTE	NRPS-1 and NRPS-3 NRPS-2 NRPS-5 PKS-4 NRPS-6 and NRPS-7
A6	GELxIxGxG (V/L) ARGYL	GELHVHHSVGMARGYW np GQIHIGGAGVAIGYV GSLWVRGNTLTRGYV GEVHIESLGITHGYW	NRPS-1 and NRPS-3 NRPS-2 NRPS-5 PKS-4 NRPS-6 and NRPS-7
A7	Y (R/K) TGD L	<i>YKTGDM</i> ?YKTDAL? YASGDL ?FDTRDL? YRTGDM	NRPS-1 and NRPS-3 NRPS-2 NRPS-5 PKS-4 NRPS-6 and NRPS-7
A8	GRxDxQVKIRGxRIELGEIE	GRQDFEVKVRGHRVDTRQVE ?GSLDVQSRIDDPRIDLCVVE? GRKDSQIKLRGYRIELGEIE ?GRMGSAIKINGCWLSPETLE? GRRDYEKVRGYRVDVRQVE	NRPS-1 and NRPS-3 NRPS-2 NRPS-5 PKS-4 NRPS-6 and NRPS-7
A9	LPxYM (I/V) P	LPTYMLP ?LPDYLLP? LPEYMLP ?LGKHHYP? LPTYMLP	NRPS-1 and NRPS-3 NRPS-2 NRPS-5 PKS-4 NRPS-6 and NRPS-7

Table 6 : Comparison of conserved sequences in A domains of peptide synthetases and in putative A domains of the Alb modules

Core	Sequences conserved in peptide synthetases*	Sequence	Alb module
A10	NGK (V/L) DR	NGK LDR ?HGRVDL? NGKVNR ?SGKVIR? NGKLDT	NRPS-1 and NRPS-3 NRPS-2 NRPS-5 PKS-4 NRPS-6 and NRPS-7

*Sourced from Marahiel *et al.*, 1997

?: non conserved sequences

np: not present

Table 7 : Comparison of conserved sequences in PCP and TE domains of peptide synthetases and in putative PCP and TE domains of the Alb modules

Domain	Sequences conserved in peptide synthetases*	Sequence	Alb module (domain)
PCP	DxFFxxLGG (H/D) S (L/I)	D-FFAVGGH SVL DNFFALGGHSL S DNFFELGGH SVL DNFFELGGHSL S DNFFNLGGHSL L	PKS-3 (PCP1) NRPS-1 and NRPS-3 (PCP2 and PCP4) NRPS-2 (PCP3) NRPS-5 (PCP5) NRPS-6 and NRPS-7 (PCP6 and PCP7)
TE	G (H/Y) SxG	GWSSG	NRPS-7

*Sourced from Marahiel *et al.*, 1997

Table 8.										
Position in GsrA (Phe) and variability										
Domains	235	236	239	278	299	301	322	330	331	517
	0	+/-	++	++	++	+/-	++	+/-	++	0
Alb NRPS-1	A	V	K	Y	V	A	N	D	A	K
Alb NRPS-3	A	V	K	Y	V	A	N	D	A	K
TyrB-M1 (Pro)	D	V	Q	S	I	A	N	V	V	K
VirS (Pro)	D	V	Q	Y	A	A	H	V	M	K
HVCL	G	A	L	H	V	V	G	S	I	K
Alb NRPS-6	A	I	K	Y	F	S	I	D	M	K
Alb NRPS-7	A	I	K	Y	F	S	I	D	M	K
VirS (Pro)	D	V	Q	Y	A	A	H	V	M	K
EntF-M1 (Ser)	D	V	W	H	F	S	L	V	D	K
β -Ala code	V	D	W	V	I	S	L	A	D	K
Alb NRPS-5	D	L	T	K	I	G	E	V	G	K
BacC-M5 (Asn)	D	L	T	K	I	G	E	V	G	K
TyrC-M1 (Asn)	D	L	T	K	I	G	E	V	G	K
Asn code	D	L	T	K	L	G	E	V	G	K

Table 9: Complementation studies of Xa23RI insertion mutants

Donor	Recipient				
	AM12	AM13	AM36	AM10	AM15
pEV639	+	+	+	-	-
pEValbXXII	+	+	+	-	-
pEVHtpG	+	+	+	-	-
pALB639	+	+	+	-	-
pUFR043	-	-	-	-	-
none	-	-	-	-	-

+ : restoration of albicidin production by alb⁻ mutant, - : no complementation. All experiments were performed at least in duplicate with at least 2 exconjugants obtained from two independent triparental conjugations.

Table 10: Albicidin production assays with *X. axonopodis* pv. *vesicatoria* exconjugants harbouring different plasmids: analysis of growth inhibition of *E. coli* DH5 α KT (susceptible to albicidin) and DH5 α Alb^rKT (resistant to albicidin) in assays performed with different antibiotic combinations (no antibiotic, tetracycline only, kanamycin only and tetracycline+kanamycin).

Bioassay medium containing	Tester strain	Combination of plasmids			
		pUFR043	pUFR043	PALB571	PALB571
		and pLAFR3	and pOp3-4/XALB2-3	and pLAFR3	and pOp3-4/XALB2-3
No antibiotic	DH5 α KT	-	-	-	-
	DH5 α Alb ^r KT	-	-	-	-
Tetracycline	DH5 α KT	-	-	-	+
	DH5 α Alb ^r KT	-	-	-	-
Kanamycin	DH5 α KT	+	+	+	+
	DH5 α Alb ^r KT	+	+	+	+
Tetracycline+kanamycin	DH5 α KT	+	+	+	+
	DH5 α Alb ^r KT	+	+	+	+

+: presence of a growth inhibition zone

All experiments were performed at least in duplicate with at least 2 exconjugants obtained from two independent triparental conjugations.

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CLAIMS

We claim:

1. A transformed host cell that comprises one or more genetic construct that comprises SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO:3.
2. The transformed host cell of claim 1, wherein said transformed host cell has been transformed with multiple genetic constructs.
3. The transformed host cell of claim 2, wherein said multiple genetic constructs contain SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO: 3, polynucleotide fragments of SEQ ID NOs: 1-3, or combinations of polynucleotide fragments of SEQ ID NOs 1-3.
4. The transformed host cell of claim 1, wherein said host cell has been transformed with one or more genetic constructs that provide a combination of polynucleotide fragments of SEQ ID NOs: 1, 2, and 3, wherein said combination of polynucleotide fragments provide a biosynthetic pathway for the production of albicidin or an albicidin-like antibiotic.
5. A genetic construct comprising at least one polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.
6. A method of making an antibiotic comprising the culturing of a transformed host cell according to claim 1, 2, 3, or 4 under conditions that allow for the production of said antibiotic.
7. The method according to claim 6, further comprising the isolation of said antibiotic.
8. An isolated or purified polynucleotide comprising:
 - (a) a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25;
 - (b) a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47;
 - (c) a polynucleotide that is complementary to a polynucleotide selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25;
 - (d) a polynucleotide that is complementary to a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47; or
 - (e) a polynucleotide that is at least 70% homologous to: (1) a polynucleotide

selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25; (2) a polynucleotide sequence encoding a polypeptide selected from the group consisting of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47; (3) a polynucleotide that is complementary to a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47; (3) a polynucleotide that is complementary to a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, and 25;

(f) a polynucleotide sequence encoding a variant of a polypeptide selected from the group consisting of SEQ ID NOs: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47, wherein said variant has at least one of the biological activities associated with the polypeptides of SEQ ID NOs: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47;

g) a polynucleotide sequence encoding a fragment of a polypeptide selected from the group consisting of SEQ ID NOs: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47 or a fragment of a variant polypeptide of SEQ ID NOs: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47;

h) a polynucleotide sequence encoding multimeric construct comprising a polynucleotide as set forth in (a), (b), (c), (d), (e), (f), or (g);

(i) a polynucleotide that hybridizes under low, intermediate or high stringency with a polynucleotide sequence as set forth in (a), (b), (c), (d), (e), (f), (g), or (h);

j) a genetic construct comprising a polynucleotide sequence as set forth in (a), (b), (c), (d), (e), (f), (g), (h), or (i);

k) a vector comprising a polynucleotide sequence as set forth in (a), (b), (c), (d), (e), (f), (g), (h), or (i); or

(l) a promoter operably linked to a polynucleotide sequence as set forth in (a), (b), (c), (d), (e), (f), (g), (h), or (i).

9. A recombinant cell comprising a polynucleotide sequence according to claim 8.

10. The recombinant cell of claim 9, wherein said cell is a plant cell.

11. The recombinant cell of claim 9, wherein said cell is bacterial.

12. The recombinant cell of claim 9, wherein said cell is eukaryotic.

13. The recombinant cell of claim 10, wherein said plant cell comprises seed

propagative materials, or plant parts.

14. A method of producing a protein comprising the steps of expressing a polynucleotide according to claim 8 in a host cell under conditions that allow for the expression of said polynucleotide.

15. The method according to claim 14, further comprising the isolation of said protein.

16. A method of producing a polyketide carrying para-aminobenzoic acid and/or carbamoyl benzoic acid by inserting at least one DNA Fragment of Claim 8 that encodes a polyketide synthetase (PKS) into a cell and causing the cell to express the encoded PKS protein under conditions such that the PKS functions to produce a polyketide carrying either a para-aminobenzoic acid or a carbamoyl benzoic acid or both.

17. A method of activating non-proteinogenic amino acids for incorporation into peptides or polyketides by inserting at least one DNA Fragment of Claim 8 that encodes a polyketide synthetase (PKS) into a cell and causing the cell to express the encoded PKS under conditions such that the PKS activates said non-proteinogenic amino acids.

18. The method according to claim 17, wherein said non-proteinogenic amino acids are paraminobenzoic acid or carbamoyl benzoic acid.

19. A polypeptide comprising:

(a) SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47;

(b) a heterologous polypeptide sequence fused, in frame, to a polypeptide comprising SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47;

(c) a fragment of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47, wherein said fragment exhibits at least one biological function of the polypeptide of SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47;

(d) a variant having at least 70% homology to a polypeptide comprising SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47, wherein said variant exhibits at least one biological function of the polypeptide comprising SEQ ID NO: 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47;

20. An isolated and purified antibiotic produced by a process that includes at least three proteins coded by DNA sequences of claim 8 in combination with additional

enzymes that modify the product to provide a non-naturally occurring Albicidin-like product having at least one of the useful properties reported for albicidin.

21. The antibiotic or antibiotics of claim 20 having at least one of the general structures illustrated in Figure 11.

22. An antibiotic produced by the process of expressing the DNA of one or more of the genes included in the Albicidin Biosynthetic Gene Clusters of Claim 8 in a genetically modified host cell sustained in a culture media, and thereafter separating the antibiotic from the host cell and culture media.

23. A process for producing an antibiotic that comprises modifying a host cell to enhance expression of a polynucleotide according to claim 8 comprising the insertion of expression enhancing DNA into the genome of a *Xanthomonas albilineans* strain, *Escherichia coli* strain, or other Albicidin producing microbial strain, in a position operative to enhance expression of the enzymes of the Albicidin Biosynthetic Gene Clusters, culturing the modified host cell to produce an antibiotic and isolating the antibiotic.

24. An isolated purified antibiotic having at least 4 of the structural elements illustrated in Figure 11, and an elemental composition of $C_{40}H_{35}N_6O_{15}$.

25. A method of protecting a plant against damage from albicidin that comprises applying an agent that blocks expression at least one gene in the Albicidin Biosynthetic Gene Clusters to the plant to be protected.

26. A method of obtaining agents useful in blocking expression of albicidin by screening materials against a modified host cell line that expresses a polynucleotide according to claim 8 and selecting for materials that stop or decrease albicidin production.

27. A method of protecting a plant against phytotoxic damage from an antibiotic that comprises inserting into the plant and operably expressing at least one resistance gene from the polynucleotides according to claim 8 in the plant to be protected.

28. The recombinant cell of claim 9, wherein said cell has been transformed with at least one polynucleotide sequence comprising SEQ ID NO: 1, 2, or 3.

29. The recombinant cell of claim 28, wherein said cell has been transformed with at least two of said polynucleotide sequences.

30. The recombinant cell of claim 28, wherein said cell has been transformed with SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

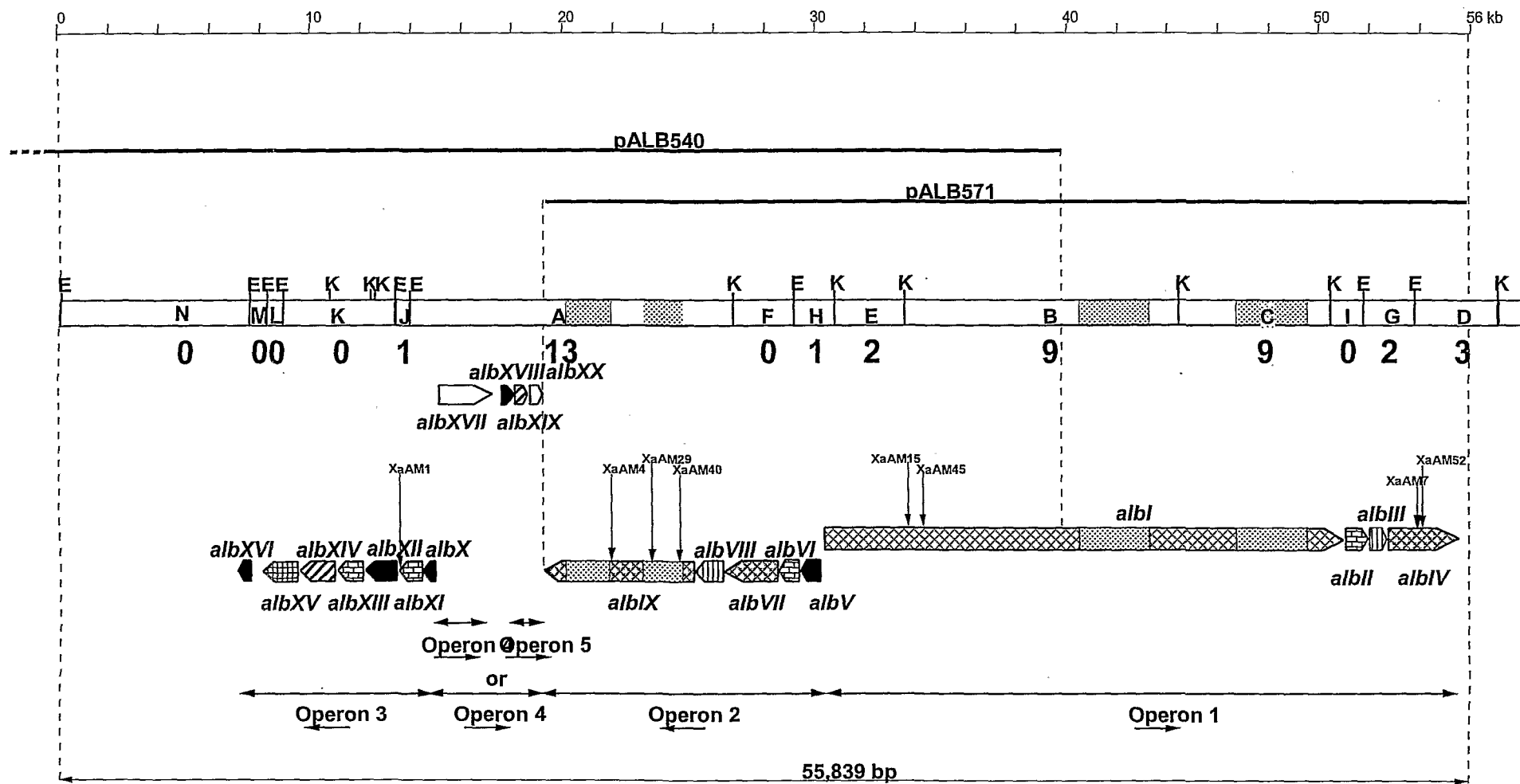


FIG. 1

AlbI

PKS-1	PKS-2	PKS-3	NRPS-1	NRPS-2	NRPS-3	NRPS-4
AL ACP1	KS1 KR ACP2 ACP3	KS2 PCP1	C A PCP2	C (A?) PCP3	C A PCP4	C

XabB

PKS-1	PKS-2	PKS-3	NRPS-1			NRPS-4
AL ACP1	KS1 KR ACP2 ACP3	KS2 PCP1	C A PCP2	-----		C

AlbIV

NRPS-5

A PCP5

AlbVII

PKS-4

HBCL

AlbIX

NRPS-6

NRPS-7

A PCP6	C A PCP7	TE
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FIG. 2

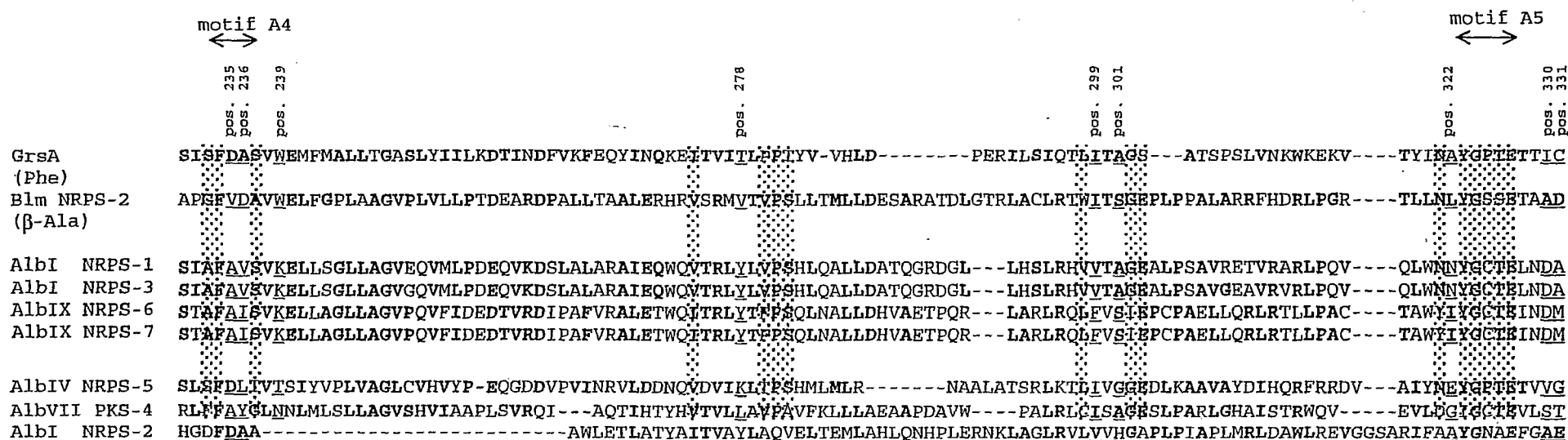
Sgl-TcmO	173	FVDLGGARG	234	PRADVFIIV	263	ALTPGGAVLV
Sgl-TcmN	331	IADLGGGDG	393	TGYDAYLF	423	IGDDDARLLI
Smy-MdmC	64	VLEIGTFTG	135	GAFDIVFV	159	LVRPGGLVAI
Mxa-SafC	63	TLEVGVFTG	134	GTFDLAFI	158	LVRPGGLIIL
Ser-EryG	85	VLDVGFGLG	149	ETFDRVTS	178	VLKPGGVLA I
Spe-DauK	183	VLDVGGGKG	254	RKADAIIL	273	ALEPGGRILI
Sal-DmpM	208	VVDIGGADG	269	GGGDLYVL	298	AMPAHARLLV
Shy-RapM	106	VLEVGC GMG	155	VQGD AEEL	194	ALRRGGALSH
Sav-AveD	71	VLDVGC GSG	124	GSEDAAWA	151	VL R PGGR L AV
Sar-Cmet	158	VLDVACGHG	220	GPYDLSLI	251	AT R PGGR IGI
AlbII	174	VLDVAAGHG	236	SGYDVILL	267	ALNDDGMVIT
		Motif I		Motif II		Motif III

FIG. 3

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Sgl-tcmP	84	VVLHLACGLDSRAFRMDV	PD109	DVDVPDVIELR	139	EDWLDTVP	150	PALVVAEGLTPYL
Sme-PKS	84	TVLHLGCGLDSRIEFRID	PGP109	ELDVDPVISLR	139	RGWIERLP	150	PTMIVAEGVLPYL
Pmu-tcmP	86	VVVQLGAGLDARFERLGK	PQ111	DLDLPEVINIR	141	TDWMKTVS	152	PVLLILEGVLMFF
Mtu-Omt	85	TVVALAEGLOTSFWRLD	VAI113	TVDLPPIVDLR	144	YSWMDSVD	155	GVFITAEGLLMYL
Mlo-Hp	84	IVLHLGCGLDTRVFRVD	PPP109	DADYPQVIELR	139	PGWLAEVP	150	PAMVVAEGLTPYL
Mtu-Hp	101	QVAILASGLDSRAYRLP	WP127	EIDQPKVMEFK	162	ADWPTALQ	178	PTAWLAEGLLIYL
Mtu-Hp2	104	QVVILASGDL SRAWRLP	WPD129	ELDQPKVLEFK	162	QDWPQALQ	178	PCAWLAEGLVRYL
Mtu-Hp3	98	QVVILAAGLDSRAYRLP	WPD123	ELDRPQVLDFK	156	DDWPQALR	172	PSAWIAEGLLIYL
Mtu-Hp4	101	QAVIVAAGLDCRAYRLD	WQP126	EIDVPKVLEFK	161	TDWPTPLT	177	PSAWSVEGLLPYL
Sco-Hp	93	QVVLLGAGMDSRAFRMA	WPE118	EVDTPAPLEFK	153	EDWPSALA	169	PTAWIGEGLLIYL
AlbVI	99	QVVILAAGMDARAYRLP	WPS124	EIDHMDVLSDK	157	EDWPQALK	173	ATLWLVEGLLCYL
		Motif I	Motif II	Motif III	Motif IV			

FIG. 4



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FIG. 5

14456 GTCGTTGATCAGCACCAAGCCTGTTCCCTCGAACGTCATCCTAAAGATACCCCGGAAGGCTGCTGCGAAGCACGGAAGTTGCTACATCGCAC
CAGCAACTAGTCTGTGGTCTATTCGGACAAGGAGCTTGCACTAGGATTTCTATGGGGGCCCTCCGACGACGCTTCGTGCCTTCAACGATGTAGCGTG
D N I L V L Y A Q E E F T M RBS
albX
-35 (PalbX: operon 3) -10 (PalbX: operon 3)
14552 AATGCGATTCAGATGGACCAAGCAAAGCGACTATACATGACGTCACTTCGAAGATGTCAAGAAAAATAGCGCGTGAAGAGCACGTAAGAGTGATGT
TTACGCTAAGTCTACCTGGTTCGTTTCGCTGATATGTACTGCAGTGAAGCTTCTACAGTTCTTTTATCGCGCACTTCTCGTGCATTCTCACTACA
-10 (PalbXVII: operon 4) -35 (PalbXVII: operon4)
14648 GTTTCGCACCGCTGTACGTCCCATCGCCATCGCGGCAAAGCTTACACGAAAAATTCACCAGGGCATGCGTTCAATACGCGGGTCAAAGCAATATCC
CAAAGCGTGGCGACATGCAGGGTAGCGGTAGCGCCGTTTCGAATGTGCTTTTAAGTGGTCCCGTACGCAAGTTATGCGCCAGTTTCGTTATAGG
14744 TTGCGCTTGCAGAGCTATGTTTCGTGCGTAAAGCGCCAAGGCAGTGGGGAGCAACACCTTGGGTTTCGGTTGAGGTGCGGGTAGCAATTTCTGCTTA
AACGCGAACGTCTCGATACAAGCACGCATTTTCGCGGTTCCGTCACCCCTCGTTGTGGAACCCAAAGCCAACCTCCACGCCCATCGTTAAAGACGAAT
RBS
14840 ATATCCACGCGCGGCGGTTTTTGTCTTGCCGGGCGTCAACTGTCTCATCGAGCAGTCTGGGAGGCTATTTTTCGCTGCCTTATCATAAATAATTAC
TATAGGTGCGCGCCGCCAAAAACAGAACGGCCCGCAGTTGACAGAGTAGCTCGTCAGACCCTCCGATAAAACGCGACGGAATAGTATTTATTAATG
M R C L I I N N Y
albXVII

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FIG. 7A

17332 GAATGAGGCCCCACGCTTACGCGCGAACCAGGACGGGCTGCTGATGGATACGGCCGGCCGGGTGGTTCGAGGGCTGCACCAGCAATCTGTTCTCG
CTTACTCCGGGGGTGCGAATGCGCGCTTGGTCCTGCCCAGGACTACCTATGCCGGCCGGCCCACCAGCTCCCGACGTGGTCGTTAGACAAGGAGC
M R P P R L R A N Q D G L
albXVIII (non expressed ?)

17428 TCGAGAACGGCCATCTGGTGACGCCCCGACCTGGGCGTGGCCGGCGTCAGCGGGATCATGCGAGGCAGGGTGATCGAATATGGCCGGCAGCACGGTC
AGCTCTTGCCGGTAGACCACCTGCGGGCTGGACCCGACCGGCCGAGTCGCCCTAGTACGCTCCGTCCCACTAGCTTATACCGGCCGTCTGTCCAG

17524 TCGCCTGCGCGGTAAAGCACGTCTATCCGGACCAGCTAGTGCGTGCTCAGGAGGTGTTTCTGACTAACGCCGTGTTTCGGCATTCTGCTGGTGCGCA
AGCGGACGCGCCATTTCTGTGCAGATAGGCCTGGTCGATCACGCACGAGTCCTCCACAAAGACTGATTGCGGCACAAGCCGTAAGACGACCACGCGT

-35 (*PalbXIX*: operon 5) -10 (*PalbXIX*: operon 5)

17620 GCATTGACGCTCACAGCTACCGCATCGATCCTGTTACCCTGCGTTTGCTCGATGCCCTGTGTTCAGGGCGTATATTTACCGAACGGTCACTACATC
CGTAACTGCGAGTGTCGATGGCGTAGCTAGGACAATGGGACGCAAACGAGCTACGGGACACAGTCCCGCATATAAAGTGGCTTGCCAGTGATGTAG

RBS

17716 AGGTTTCCACCCATGCCGGCCAAGACCCTTGAAAGCAAGGATTACTGTGGAGAAAGCTTCGTCAGCGAAGATCGCTCCGGGCAATCGCTGGAGTCG
TCCAAAGGTGGGTACGGCCGGTTCTGGGAACTTTCGTTCCCTAATGACACCTCTTTCGAAGCAGTCGCTTCTAGCGAGGCCCGTTAGCGACCTCAGC
M P A K L E S K D Y C G E S F V S
albXIX

FIG. 7B

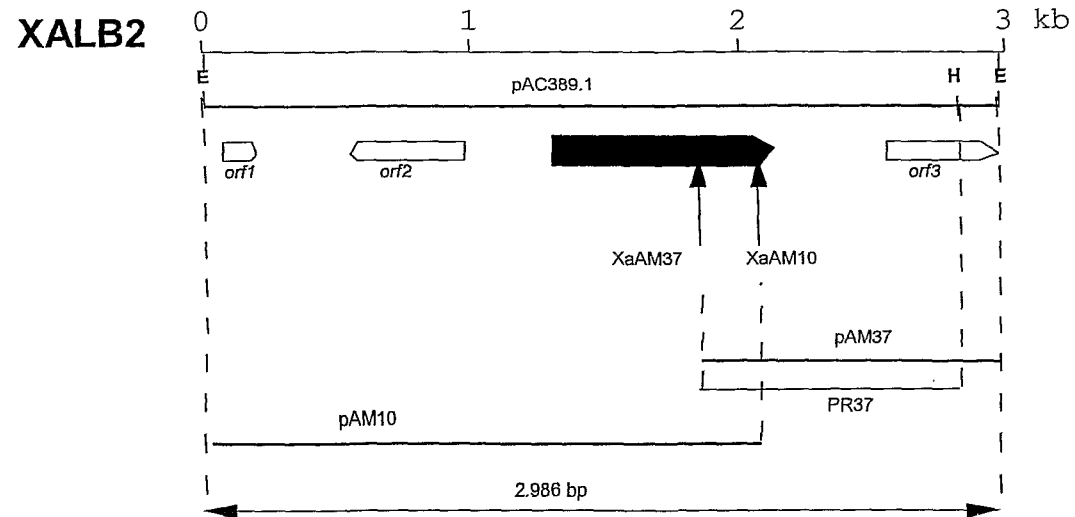
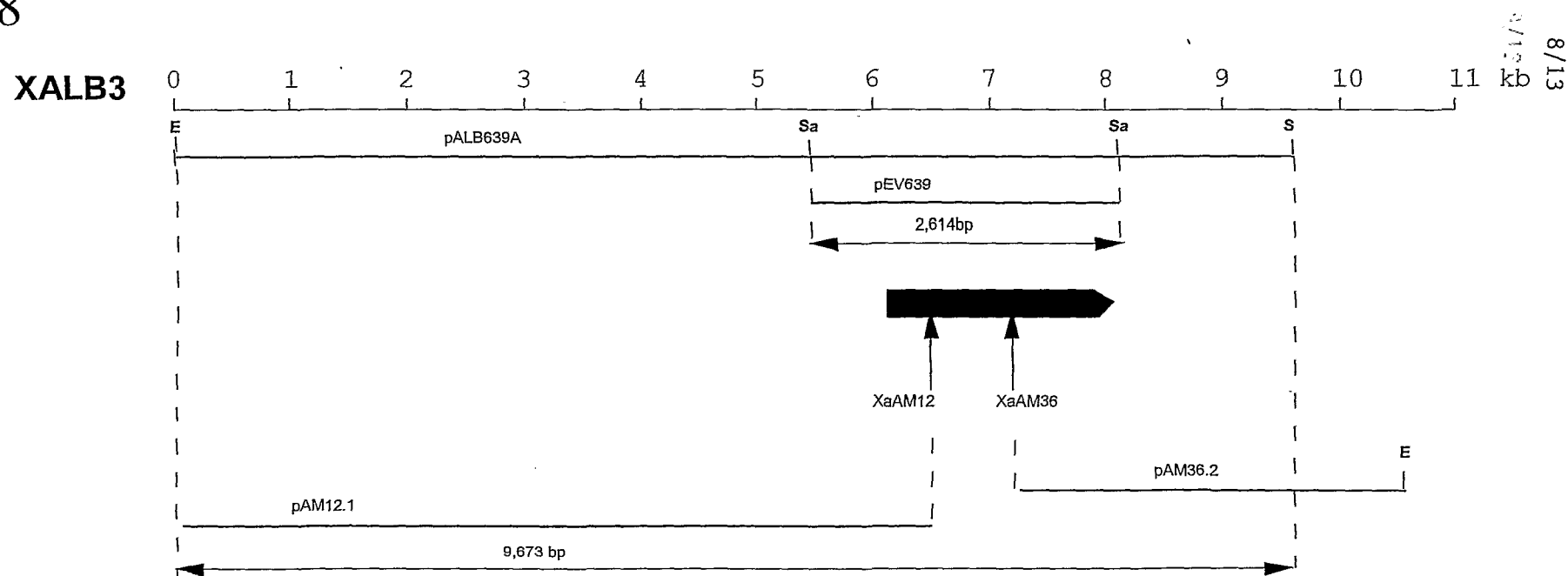


FIG. 8

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8/13 kb

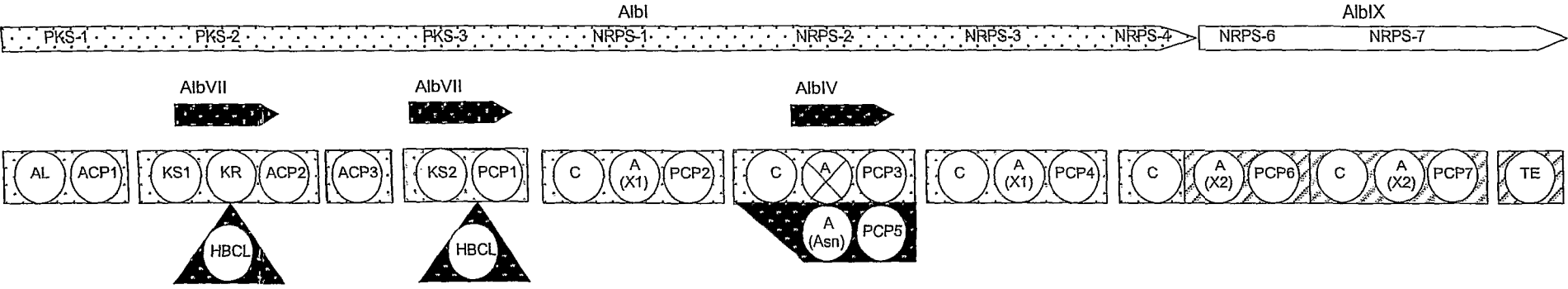


FIG. 9A

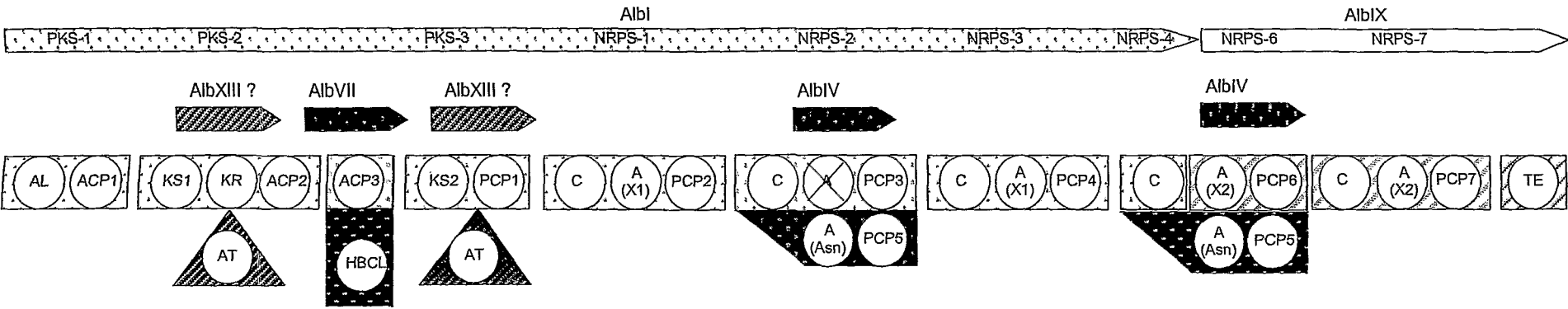


FIG. 9B

RifA-1 LGRVDVLQPAC**FA**VMVGLAAVWESV**GVR**PDVV**GHS**QGEI
RifA-2 LDQTMYTQGAL**FA**VETALFRLFESW**GVR**PGLLAG**HS**IGEL
RifA-3 LDRVDVVQPAS**FA**MMVGLAAVWTS**LGVT**PDVVL**GHS**QGEI
RifB-1 LDRVDVVQPAS**FA**VMVGLAAVWESV**GVR**PDVV**GHS**QGEI
RifE-1 LNQTVFTGAGL**FA**VESALFRLAESW**GVR**PDVVL**GHS**IGEI
BlmVIII ADDTRAAQPAL**FA**VEYALARTLMDW**GVR**PAAML**GHS**LGEV

FIG. 10A

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AlbXIII LEDRPRHIRAVIDTLTGHAQFGPAIQAHNVAV**IGH**SVGGY
FenF TRTMNAQPAILTVSVIAYQVYMQE**IGIK**PHFLAG**HS**LGEY
LipA PDSRGRQLLAALDYLTGRSSVRGRIDSGRLGVM**GHS**MGGG

FIG. 10B

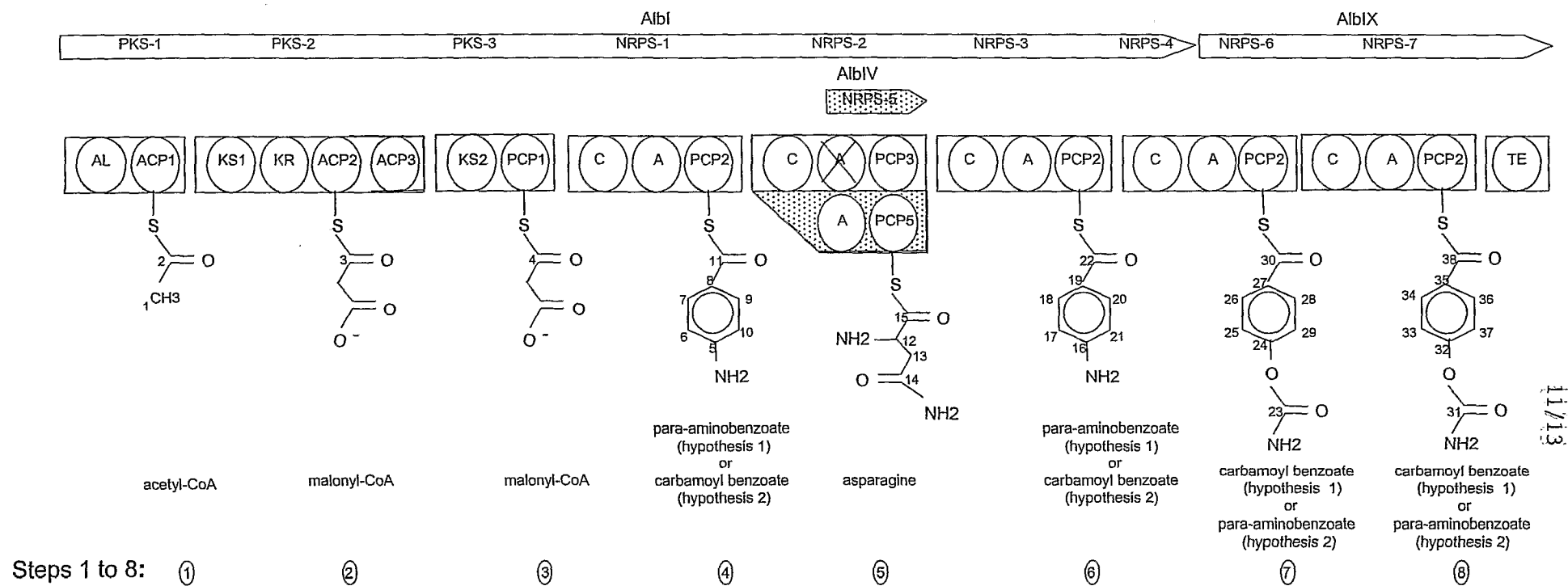
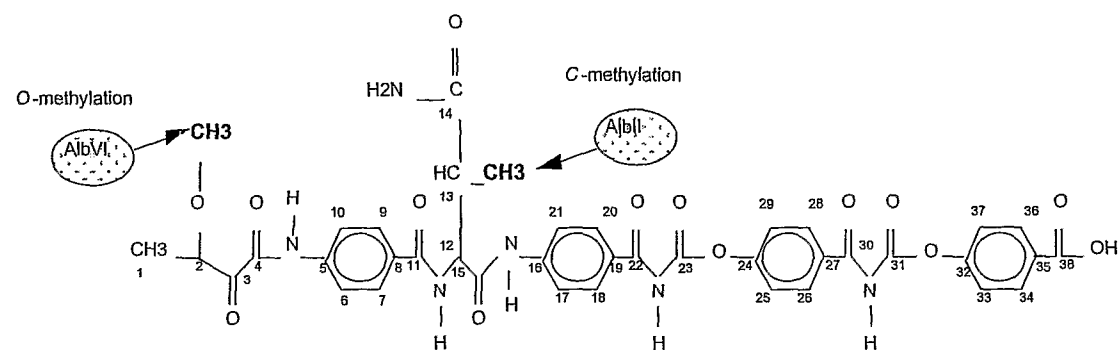


FIG. 11A



$C_{40}O_{15}N_6H_{35}$
(molecular weight : 839)

or

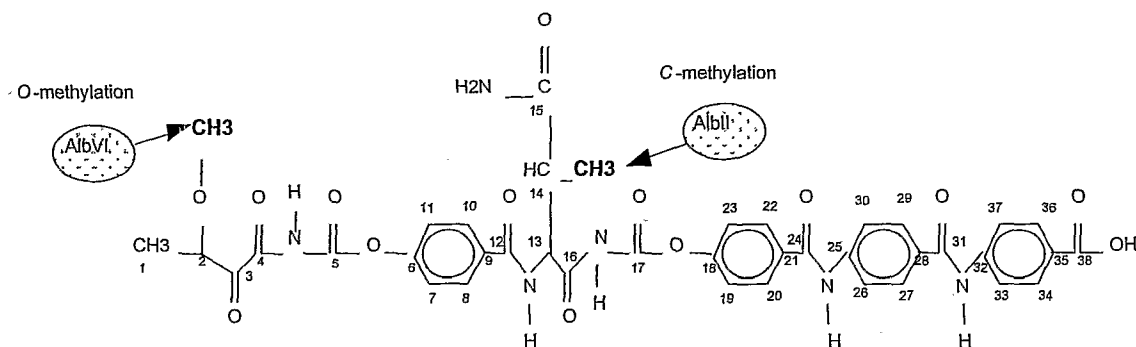


FIG. 11B

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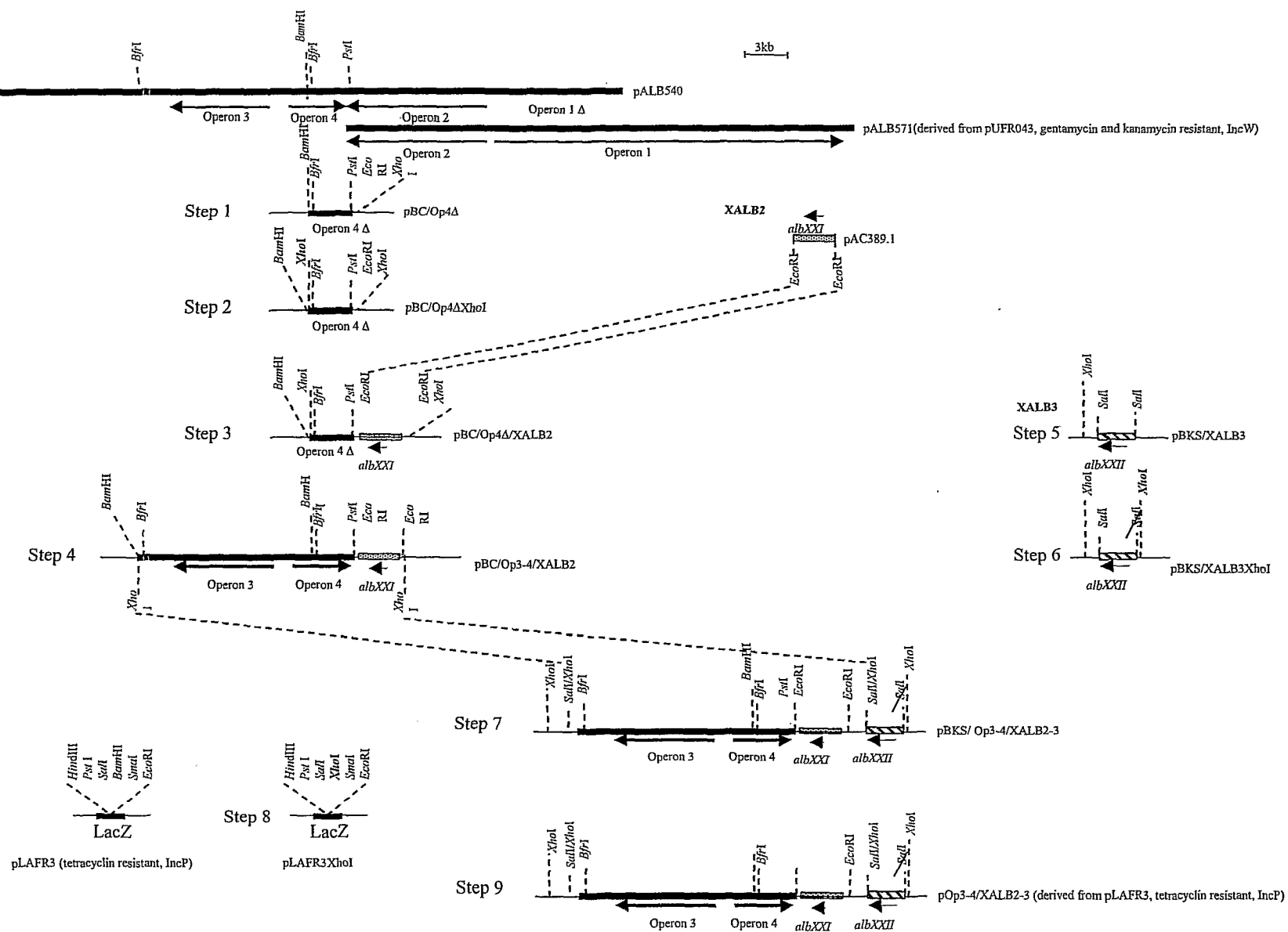


FIG. 12

SEQUENCE LISTING

<110> University of Florida and Centre de Coopération Internationale
en Recherche Agronomique pour le Développement (CIRAD)

<120> Complete Biosynthetic Gene Set for Synthesis of Polyketide
Antibiotics, Including the Albicidin Family, Resistance Genes, and
Uses Thereof

<130> UF-398XC1

<150> US 60/419,463

<151> 2002-10-18

<160> 54

<170> PatentIn version 3.1

<210> 1

<211> 55839

<212> DNA

<213> *Xanthomonas albilineans*

<400> 1

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ggcttgtgtg	tcggtctgca	tcagttcgcg	gatgctatcg	cgccgaaggc	caccgcgttg	120
gctactccgg	tgcggcagat	tggagcgatg	ggacatgcac	ccgctagcgc	ggccagtgcg	180
ccgatgactg	gcggggtgtt	cgtctctttt	tcgcgaacgc	cgggtgcgatt	gcccgcggta	240
acaggcatga	aggtttctaa	tgagggccat	gacggcatgg	cgggcgcggc	tatcactcat	300
gcggcggtaa	aaccggtgat	ggctccattg	acaggggaca	tgttgacagg	cgcggtgagc	360
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<210> 6

<211> 1491

<212> DNA

<213> Xanthomonas albilineans

<400> 6

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<210> 7

<211> 954

<212> DNA

<213> Xanthomonas albilineans

<400> 7

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<210> 8

<211> 1356

<212> DNA

<213> *Xanthomonas albilineans*

<400> 8

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<210> 9

<211> 948

<212> DNA

<213> *Xanthomonas albilineans*

<400> 9

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ccgtccatcg	gtagtgcagt	attgcgacag	ccttaccctt	gcgagcgcg	cgtccagggc	900
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<210> 10

<211> 252

<212> DNA

<213> Xanthomonas albilineans

<400> 10

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catgcactcg	tccttaccgc	cagcatagcc	ttccttgccg	cagccaggcg	ggacctcaag	180
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<210> 11

<211> 2151

<212> DNA

<213> Xanthomonas albilineans

<400> 11

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<210> 12

<211> 414

<212> DNA

<213> Xanthomonas albilineans

<400> 12

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<210> 13

<211> 603

<212> DNA

<213> Xanthomonas albilineans

<400> 13

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<210> 14

<211> 609

<212> DNA

<213> Xanthomonas albilineans

<400> 14

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<210> 15

<211> 5880

<212> DNA

<213> Xanthomonas albilineans

<400> 15

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<213> Xanthomonas albilineans

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<211> 837

<212> DNA

<213> Xanthomonas albilineans

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ccggaactgc	aggcagccgc	gcgcagtgc	caccgccatc	tgctcgacga	cggcacggcg	180
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atcgcccgcc	cgacagcat	cgcccgagc	gtgcgcaagc	gtcaggccga	gttcctgttc	300
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gacattgcaa	tcggcgcgac	gcgcgcgcc	tgctggcctg	ccggcagcct	gggcagcatt	420
tcccatttgc	aggactacgc	ggccgccatc	gccatggcgg	ccggcaccgc	ccacggcggtg	480
ggcatcgatc	tggaacgacc	aatcacaccc	gcggcgcgcg	cggtgttgct	gagcatcgca	540
atcgatgccc	acgaagccgc	tcgtctggca	aaggcggcag	acgcgcagtg	gccgcaagac	600
ctgctgctga	ccgcaactat	ttcggccaa	gaaagcctgt	tcaaagccgc	ctacagcgcg	660
gtcggacgct	acttcgactt	cagcgcgcca	cgctgtgctg	gcacgcagct	ggcacggcaa	720
tgcttgcata	tgccgctgac	cgagacactc	tgccgcgaat	tcgtggccgg	gcaagtgtgc	780
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<210> 25

<211> 1905

<212> DNA

<213> Xanthomonas albilineans

<400> 25

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gacggcgatg	cgcaactgcg	catccgcata	ggcttcgaca	aggacgcccg	caccgtcacc	240
atcgacgaca	acggcatcgg	catgagccgc	gaggagatcg	tcgcgcacct	gggcaccatc	300
gccaaatccg	gcacctccga	tttccctcaag	catctgtccg	gcgatcagaa	gaaggattcg	360
cacctgatcg	gccagttcgg	tgctggcttc	tacagtgcct	tcacgtcgcg	cgatcaagtg	420
gacgtgtaca	gccgtcgcgc	cggtctgccc	gccagcgacg	gcgtacactg	gtcctcgcgt	480
ggcgaaggcg	agttcgaggt	cgccaccatc	gacaagcccg	agcgcgccac	ccgcacgtgtg	540
ctgcacttga	aggagggaaga	gaaaggcttc	gcgcagcggt	ggaagttgcg	cagcatcggtg	600
cgcaagtact	ccgaccacat	cgcttgcgcg	atcgagctaa	tcaagggaaca	ctacggcgag	660
gacaaggaca	agccggaaac	ccccgagtg	gagacgtca	atcgcgccag	cgcgctgtgg	720
acacggccgc	gcaccgagat	caaggacgag	gaataccaag	aactgtacaa	gcacattgcc	780
cacgaccacg	aaaaccgggt	ggcgtggagc	cataacaagg	tcgaaggcaa	actggaatac	840

```

acctcgtctgc tgtacctgcc cggccgcgcc ccgttcgacc tgtaccagcg cgatgcctcg 900
cgcggggtca agctgtacgt gcagcgcgtc ttcacatcatgg accaggccga ccaattcctg 960
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cgcgcactgg acatgctgga aaagctcgcc aaagacgatc ccgaacgcta caagggcgtg 1140
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aagatcgccg gcctgctgcg cttcgcgtcc acccacagcg gcgacgacgc ccagaacgtg 1260
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ggggaaaagct acgcgcaaat caaggacagc ccgcacctgg aggtgttccg caagaagggc 1380
atcgaggtgc tcctgctcac cgaccgcacg gacgagtggc tgatgagcta tctcaccgag 1440
ttcgacagca aatccttcgt cgatgtggcg cgcggcgacc tggacctggg caagctggac 1500
agcgaagaag aaaagcaggg gcaggaagaa gccgccaagg ccaagcaagg gctggccgag 1560
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catccgctga tcgagaaact ggatgcggaa cccgatgtcg atcgtttcgg tgatctggcg 1800
cggtgtgtgt tcgatcaggc cgcgctggcc gccggcgaca gcctcaagga cccggccgcc 1860
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<210> 26

<211> 6879

<212> PRT

<213> Xanthomonas albilineans

<400> 26

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Met Pro Asn Ala Leu Met Gln Ile Thr Leu Val Ala Val Gln Phe Ala
1           5           10           15

```

```

Gly Val Leu Leu Gly Val Thr Ala Arg Ala Ala Ile Pro Asn Lys Ala
20           25           30

```

```

Gly Met Arg Arg Ala Trp Pro Pro Phe Pro Gln Ala Cys Cys Arg Ser
35           40           45

```

```

Ile Ala Tyr Leu Met Gln Arg Ser Pro Met Ser Pro Leu Gln Gln Thr
50           55           60

```

```

Leu Leu Thr Arg Leu Ala Ser Ala Ala Ala Ser Arg Thr Met Ile Glu
65           70           75           80

```

```

Phe Pro Arg Pro Glu His Ala Ser Pro Gln Cys Cys Asp Asp Ala Glu
85           90           95

```

```

Leu Ala Arg Leu Ile Val Gln Leu Ser Ala Gly Leu Gln Pro Leu Ala
100          105          110

```

```

Met Pro Gly Thr Tyr Val Ile Ile Ala Ala Pro His Gly Gly Leu Phe
115          120          125

```

```

Ala Ala Ala Leu Leu Ala Cys Leu His Ala Asn Leu Val Ala Val Pro
130          135          140

```

```

Phe Pro Leu Asp Val Ala Gln Pro Asn Glu Arg Glu Gln Ala Arg Leu
145          150          155          160

```

```

Glu Thr Ile His Ala Gln Leu Met Glu His Gly Asn Val Ala Val Leu
165          170          175

```

```

Leu Asp Asp Val Ala Asp Arg Ser Ala Phe Ala Arg Met Ala His Ala

```

180					185					190					
Ala	Gly	Thr	Phe	Leu	Ala	Thr	Phe	Ala	Asp	Leu	Lys	Arg	Glu	Ser	Thr
	195						200					205			
Ser	Ala	Ser	Leu	Cys	Pro	Ala	Ser	Pro	Ser	Asp	Ala	Ala	Leu	Leu	Leu
	210					215					220				
Phe	Thr	Ser	Gly	Ser	Ser	Gly	Glu	Ser	Lys	Gly	Ile	Leu	Leu	Ser	His
225						230					235				240
Arg	Asn	Leu	His	His	Gln	Ile	Gln	Ala	Gly	Ile	Arg	Gln	Trp	Ser	Leu
				245					250					255	
Asp	Glu	His	Ser	His	Val	Val	Thr	Trp	Leu	Ser	Pro	Ala	His	Asn	Phe
			260					265					270		
Gly	Leu	His	Phe	Gly	Leu	Leu	Ala	Pro	Trp	Phe	Ser	Gly	Ala	Thr	Val
	275						280					285			
Ser	Phe	Ile	His	Pro	His	Ser	Tyr	Met	Lys	Arg	Pro	Gly	Phe	Trp	Leu
	290					295					300				
Glu	Thr	Val	Ala	Ala	Arg	Asp	Ala	Thr	His	Met	Ala	Ala	Pro	Asn	Phe
305						310					315				320
Ala	Phe	Asp	Tyr	Cys	Cys	Asp	Trp	Val	Met	Val	Glu	Gln	Leu	Pro	Pro
				325					330					335	
Ser	Ala	Leu	Ser	Thr	Leu	Thr	His	Ile	Val	Cys	Gly	Gly	Glu	Pro	Val
			340				345						350		
Arg	Ala	Ser	Thr	Met	Gln	Arg	Phe	Phe	Glu	Lys	Phe	Ala	Gly	Leu	Gly
		355					360					365			
Ala	Arg	Thr	Gln	Thr	Phe	Met	Pro	His	Phe	Gly	Leu	Ser	Glu	Thr	Gly
	370					375					380				
Ala	Leu	Ser	Thr	Leu	Asp	Glu	Ala	Pro	Gln	Gln	Arg	Val	Leu	Glu	Leu
385						390					395				400
Asp	Ala	Asp	Ala	Leu	Asn	Lys	Arg	Lys	Arg	Val	Ala	Ala	Gly	Ala	Ser
				405					410					415	
Gln	Ala	Arg	Val	Thr	Val	Leu	Asn	Cys	Gly	Ala	Val	Asp	Gln	Asp	Val
			420					425					430		
Glu	Leu	Arg	Ile	Val	Cys	Pro	Glu	Gly	Glu	Thr	Leu	Cys	Arg	Pro	Asp
		435					440					445			
Glu	Ile	Gly	Glu	Ile	Trp	Val	Lys	Ser	Pro	Ala	Ile	Ala	Arg	Gly	Tyr
	450					455					460				
Leu	Phe	Ala	Lys	Pro	Ala	Asp	Gln	Arg	Gln	Phe	Asn	Cys	Ser	Ile	Arg
465						470					475				480
His	Thr	Asp	Asp	Ser	Gly	Tyr	Phe	Arg	Thr	Gly	Asp	Leu	Gly	Phe	Ile
				485					490					495	
Ala	Asp	Gly	Cys	Leu	Tyr	Val	Thr	Gly	Arg	Val	Lys	Glu	Val	Leu	Ile
			500					505					510		

Ile Arg Gly Lys Asn His Tyr Pro Ala His Ile Glu Ala Ser Ile Ala
 515 520 525
 Ala Thr Ala Ser Pro Gly Ala Leu Met Pro Val Val Phe Ser Ile Glu
 530 535 540
 Arg Gln Asp Glu Glu Arg Val Ala Ala Val Ile Ala Val Asn His Pro
 545 550 555 560
 Trp Thr Pro Ala Ala Cys Ala Ala Gln Ala His Lys Ile Arg Gln Gln
 565 570 575
 Val Ala Asp Gln His Gly Val Ala Leu Ala Glu Leu Ala Phe Ala Glu
 580 585 590
 His Arg His Val Phe Gly Thr Tyr Pro Gly Lys Leu Lys Arg Arg Leu
 595 600 605
 Val Lys Glu Ala Tyr Val Asn Gly Gln Leu Pro Leu Leu Trp His Glu
 610 615 620
 Gly Lys Asn Arg Asp Val Pro Ala Ala Ala Ala Asp Asp Arg Gln Ala
 625 630 635 640
 Gln His Val Ala Asp Leu Cys Arg Lys Val Phe Leu Pro Val Leu Gly
 645 650 655
 Val Ala Pro Pro His Ala Gln Trp Pro Leu Cys Glu Leu Ala Leu Asp
 660 665 670
 Ser Leu Gln Cys Val Arg Leu Ala Gly Ala Ile Glu Glu Cys Tyr Gly
 675 680 685
 Val Pro Phe Glu Pro Thr Leu Leu Phe Lys Leu Glu Thr Val Gly Ala
 690 695 700
 Ile Ala Glu Tyr Val Leu Ala His Gly Arg Gln Ala Pro Thr Pro Thr
 705 710 715 720
 Arg Ala Pro Val Ala Ser Thr Thr Cys Ser Glu Glu Pro Ile Ala Ile
 725 730 735
 Val Ala Met His Cys Glu Val Pro Gly Ala Gly Glu Asn Thr Glu Ala
 740 745 750
 Leu Trp Ser Phe Leu Arg Ser Asp Val Asn Ala Ile Arg Pro Ile Glu
 755 760 765
 Ser Thr Arg Pro Asp Leu Trp Ala Ala Met Arg Ala Tyr Pro Gly Leu
 770 775 780
 Ala Gly Glu Gln Leu Pro Arg Tyr Ala Gly Phe Leu Asp Asp Val Asp
 785 790 795 800
 Ala Phe Asp Ala Ala Phe Phe Gly Ile Ser Arg Arg Glu Ala Glu Cys
 805 810 815
 Met Asp Pro Gln Gln Arg Lys Val Leu Glu Met Val Trp Lys Leu Ile
 820 825 830
 Glu Gln Ala Gly His Asp Pro Leu Ser Trp Gly Gly Gln Pro Val Gly

835	840	845
Leu Phe Val Gly Ala His Thr Ser Asp Tyr Gly Glu Leu Leu Ala Ser		
850	855	860
Gln Pro Gln Leu Met Ala Gln Cys Gly Ala Tyr Ile Asp Ser Gly Ser		
865	870	875
His Leu Thr Met Ile Pro Asn Arg Ala Ser Arg Trp Phe Asn Phe Thr		
	885	890
Gly Pro Ser Glu Val Ile Asn Ser Ala Cys Ser Ser Ser Leu Val Ala		
	900	905
Leu His Arg Ala Val Gln Ser Leu Arg Gln Gly Glu Ser Ser Val Ala		
	915	920
Leu Val Leu Gly Val Asn Leu Ile Leu Ala Pro Lys Val Leu Leu Ala		
	930	935
Ser Ala Ser Ala Gly Met Leu Ser Pro Asp Gly Arg Cys Lys Thr Leu		
945	950	955
Asp Ala Ala Ala Asp Gly Phe Val Arg Ser Glu Gly Ile Ala Gly Val		
	965	970
Ile Leu Lys Pro Leu Ala Gln Ala Leu Ala Asp Gly Asp Arg Val Tyr		
	980	985
Gly Leu Val Arg Gly Val Ala Val Asn His Gly Gly Arg Ser Asn Ser		
	995	1000
Leu Arg Ala Pro Asn Val Asn Ala Gln Arg Gln Leu Leu Ile Arg		
	1010	1015
Thr Tyr Gln Glu Ala Gly Val Glu Pro Ala Ser Val Gly Tyr Val		
	1025	1030
Glu Leu His Gly Thr Gly Thr Ser Leu Gly Asp Pro Ile Glu Ile		
	1040	1045
Gln Ala Leu Lys Glu Ala Phe Ile Ala Leu Gly Ala Gln Ala Ala		
	1055	1060
Pro Ser Asn Cys Gly Ile Gly Ser Val Lys Ser Ala Leu Gly His		
	1070	1075
Leu Glu Ala Ala Ala Gly Leu Thr Gly Leu Ile Lys Val Leu Leu		
	1085	1090
Met Leu Lys His Gly Glu Gln Ala Gly Thr Arg His Phe Ser Thr		
	1100	1105
Leu Asn Pro Leu Ile Asp Leu Arg Gly Thr Ser Phe Glu Val Val		
	1115	1120
Ala Gln His Arg Ala Trp Pro Ser Gln Val Gly Ile His Gly Thr		
	1130	1135
Leu Leu Pro Arg Arg Ala Gly Ile Ser Ser Phe Gly Phe Gly Gly		
	1145	1150

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1460		1465		1470
Glu Asp Cys Tyr Ala His Phe Thr Ala Cys Gly Leu Gln Leu Gly				
1475		1480		1485
Asp Arg Leu Lys Ser Val Gln Ser Ile Gly Cys Gly Arg Asn Gly				
1490		1495		1500
Glu Gly Glu Pro Ile Ala Leu Gly Val Leu Arg Leu Pro Pro Ser				
1505		1510		1515
Ser Val Glu Asp Ser His Val Leu Pro Pro Ser Leu Leu Asp Gly				
1520		1525		1530
Ala Leu Gln Cys Ser Leu Gly Leu Gln Arg Asp Val Glu His Ile				
1535		1540		1545
Ala Met Pro Tyr Thr Leu Glu Arg Met Thr Val His Ala Pro Ile				
1550		1555		1560
Pro Pro Glu Ala Trp Val Leu Leu Arg His Gly His Ala Ala Arg				
1565		1570		1575
Gln Ser Leu Asp Ile Asp Leu Leu Asp Ser Glu Gly Arg Val Cys				
1580		1585		1590
Val Ser Leu Gly Asn Tyr Thr Gly Arg Ala Pro Lys Ala Val Ser				
1595		1600		1605
Ala Val Arg Ala Leu Val Leu Ala Pro Val Trp Gln Ala Leu Thr				
1610		1615		1620
Glu Thr Ala Pro Ala Trp Pro Asp Pro Ala Glu Arg Ile Val Thr				
1625		1630		1635
Val Gly Asp Asp Ala Trp Arg Ser His Phe Gly Phe Asp Glu Pro				
1640		1645		1650
Ala Leu Ser Leu Glu Asp Ser Val Glu Val Ile Ala Thr Arg Leu				
1655		1660		1665
Gly Gln Ser Gly Lys Phe Asp His Leu Val Trp Ile Val Pro Ile				
1670		1675		1680
Ala Glu Ser Glu Thr Asp Ile Ala Ala Gln Gly Ser Ala Ala Ile				
1685		1690		1695
Ala Gly Phe Arg Leu Val Lys Ala Leu Leu Ala Leu Gly Tyr Ala				
1700		1705		1710
His Arg Pro Leu Gly Leu Thr Val Leu Thr Arg Gln Ala Leu Thr				
1715		1720		1725
Arg Gln Pro Ser His Ala Ala Val His Gly Leu Ile Gly Thr Leu				
1730		1735		1740
Ala Lys Glu Tyr Cys Asn Trp Lys Ile Arg Leu Leu Asp Leu Pro				
1745		1750		1755
Ser Val Lys Ser Trp Pro Gln Trp Glu Gln Leu Arg Ser Leu Pro				
1760		1765		1770

Trp	His	Ala	Gln	Gly	Glu	Ala	Leu	Ile	Gly	Arg	Gly	Thr	Cys	Trp
1775						1780					1785			
Tyr	Arg	Arg	Gln	Leu	Cys	Glu	Val	Leu	Pro	Leu	Pro	Ser	Leu	Glu
1790						1795					1800			
Pro	Pro	Pro	Tyr	Arg	Val	Gly	Gly	Val	Tyr	Val	Val	Ile	Gly	Gly
1805						1810					1815			
Ala	Gly	Gly	Leu	Gly	Glu	Val	Leu	Ser	Glu	His	Leu	Ile	Arg	Thr
1820						1825					1830			
Tyr	Asp	Ala	Gln	Leu	Ile	Trp	Ile	Gly	Arg	Arg	Val	Leu	Asp	Glu
1835						1840					1845			
Gly	Ile	Ala	Arg	Lys	Gln	Thr	Arg	Leu	Ala	Ser	Leu	Gly	Arg	Ala
1850						1855					1860			
Pro	His	Tyr	Ile	Ser	Ala	Asp	Ala	Ser	Asp	Pro	Ala	Ala	Leu	Gln
1865						1870					1875			
Ala	Ala	His	Asn	Glu	Ile	Val	Ala	Leu	His	Gly	Gln	Pro	His	Gly
1880						1885					1890			
Leu	Ile	Leu	Ser	Asn	Ile	Val	Leu	Lys	Asp	Ala	Ser	Leu	Ala	Arg
1895						1900					1905			
Met	Glu	Glu	Ala	Asp	Phe	Arg	Asp	Val	Leu	Ala	Ala	Lys	Leu	Asp
1910						1915					1920			
Val	Ser	Val	Cys	Ala	Ala	Gln	Val	Phe	Gly	Thr	Ala	Pro	Leu	Asp
1925						1930					1935			
Phe	Val	Leu	Phe	Phe	Ser	Ser	Ile	Gln	Ser	Thr	Thr	Lys	Ala	Ala
1940						1945					1950			
Gly	Gln	Gly	Asn	Tyr	Ala	Ala	Gly	Cys	Cys	Tyr	Val	Asp	Ala	Phe
1955						1960					1965			
Gly	Glu	Leu	Trp	Ala	Arg	Arg	Gly	Leu	Arg	Val	Lys	Thr	Ile	Asn
1970						1975					1980			
Trp	Gly	Tyr	Trp	Gly	Ser	Val	Gly	Val	Val	Ala	Gly	Glu	Asp	Tyr
1985						1990					1995			
Arg	Arg	Arg	Met	Ala	Gln	Lys	His	Met	Ala	Ser	Ile	Glu	Gly	Ala
2000						2005					2010			
Glu	Ala	Met	Gln	Val	Leu	Ser	Gln	Leu	Leu	Cys	Ala	Pro	Leu	Gln
2015						2020					2025			
Arg	Leu	Ala	Tyr	Val	Lys	Ile	Asp	Asp	Ala	Asn	Ala	Met	Arg	Ala
2030						2035					2040			
Leu	Gly	Val	Val	Glu	Asp	Glu	Ser	Val	Gln	Ile	Pro	Val	His	Ala
2045						2050					2055			
Pro	Ala	Glu	Pro	Pro	Arg	Gly	Gln	Pro	Gly	Pro	Val	Val	Glu	Leu
2060						2065					2070			

Ser	Val	Asn	Leu	Asp	Ala	Arg	Arg	Glu	Arg	Glu	Thr	Leu	Leu	Ala
	2075					2080					2085			
Ala	Trp	Leu	Leu	Glu	Leu	Ile	Glu	Gln	Leu	Gly	Gly	Phe	Pro	Pro
	2090					2095					2100			
Ala	Ser	Phe	Asp	Ile	Ala	Thr	Leu	Ala	Gln	Arg	Leu	His	Ile	Val
	2105					2110					2115			
Pro	Ala	Tyr	Arg	Ser	Trp	Leu	Glu	His	Ser	Val	Arg	Met	Leu	Gly
	2120					2125					2130			
Val	Tyr	Gly	Tyr	Leu	Arg	Ala	Thr	Gly	Glu	Ser	Arg	Phe	Glu	Leu
	2135					2140					2145			
Ala	Asp	Lys	Pro	Pro	Asp	Asp	Ala	Arg	Gly	Ala	Trp	Asn	Ala	His
	2150					2155					2160			
Val	His	Glu	Ala	Ser	Val	Glu	Ala	Gly	Glu	Glu	Ala	Gln	Arg	Arg
	2165					2170					2175			
Leu	Leu	Asp	Arg	Cys	Met	Arg	Ala	Leu	Pro	Ala	Val	Leu	Arg	Gly
	2180					2185					2190			
Glu	Arg	Lys	Ala	Thr	Glu	Leu	Leu	Phe	Pro	Glu	Gly	Ser	Met	Ala
	2195					2200					2205			
Trp	Val	Glu	Gly	Ile	Tyr	Gln	Asn	Asn	Pro	Leu	Ala	Asp	Tyr	Phe
	2210					2215					2220			
Asn	Ala	Gln	Leu	Val	Thr	Arg	Leu	Ile	Ala	Tyr	Leu	Arg	Arg	Arg
	2225					2230					2235			
Leu	Glu	Ser	Thr	Pro	Thr	Ala	Arg	Leu	Lys	Leu	Cys	Glu	Ile	Gly
	2240					2245					2250			
Ala	Gly	Ser	Gly	Gly	Thr	Thr	Ala	Ser	Val	Leu	Gln	Gln	Leu	Gln
	2255					2260					2265			
Ala	Tyr	Gly	Glu	His	Ile	Glu	Glu	Tyr	Leu	Tyr	Thr	Asp	Leu	Ser
	2270					2275					2280			
Pro	Val	Phe	Leu	His	His	Ala	Glu	Lys	His	Tyr	Gln	Pro	Arg	Ala
	2285					2290					2295			
Pro	Tyr	Leu	Arg	Thr	Ala	Cys	Phe	Asp	Val	Ala	Arg	Ala	Pro	Thr
	2300					2305					2310			
Ala	Gln	Ala	Leu	Glu	Ser	Gly	Gly	Tyr	Asp	Val	Val	Ile	Ala	Ala
	2315					2320					2325			
Asn	Val	Leu	His	Ala	Thr	Arg	Asp	Ile	Ala	Lys	Thr	Leu	Arg	Asn
	2330					2335					2340			
Ala	Lys	Ala	Leu	Leu	Lys	Pro	Gly	Gly	Leu	Leu	Leu	Leu	Asn	Glu
	2345					2350					2355			
Val	Ile	Glu	Arg	Ser	Leu	Val	Leu	His	Leu	Thr	Phe	Gly	Leu	Leu
	2360					2365					2370			
Glu	Ser	Trp	Trp	Leu	Pro	Gln	Asp	Lys	Ile	Leu	Arg	Leu	Ala	Gly

2375	2380	2385
Ser Pro Leu Leu Ala Cys	Ala Thr Trp Arg Ser	Leu Leu Glu Ala
2390	2395	2400
Glu Gly Phe Ala Gly Leu	Ser Val His Arg Ala	Gln Pro Asp Ala
2405	2410	2415
Gly Gln Ala Ile Ile Cys	Ala Tyr Ser Asp Gly	Ile Val Arg Gln
2420	2425	2430
Ala Ser Thr Ile Glu Val	Ala Arg Asn Glu Lys	Val Thr Val Pro
2435	2440	2445
Ser Gln Pro Ala Glu Ala	Gly Glu Ser Pro Leu	Asp Leu Val Lys
2450	2455	2460
Lys Leu Leu Gly Arg Ile	Leu Lys Met Asp Pro	Ala Thr Leu Asp
2465	2470	2475
Thr Ser His Pro Leu Glu	Tyr Tyr Gly Val Asp	Ser Ile Val Ala
2480	2485	2490
Ile Glu Leu Ala Met Ala	Leu Arg Glu Thr Phe	Pro Gly Phe Glu
2495	2500	2505
Val Ser Glu Leu Phe Glu	Thr Gln Ser Ile Asp	Thr Leu Leu Gly
2510	2515	2520
Ser Leu Glu Gln Ala Pro	Leu Leu Ala Thr Leu	Thr Ala Pro Pro
2525	2530	2535
Gln Gln Asp Met Leu Gln	Gln Leu Lys Gln Leu	Leu Ala Arg Thr
2540	2545	2550
Leu Lys Leu Asp Ile Thr	Gln Ile Asp Thr Ser	Lys Thr Leu Glu
2555	2560	2565
Ser Tyr Gly Val Asp Ser	Ile Val Ile Ile Glu	Leu Ala Asn Ala
2570	2575	2580
Leu Arg Glu Arg Tyr Pro	Ser Leu Asp Ala Ser	Gln Leu Met Glu
2585	2590	2595
Thr Leu Ser Ile Asp Arg	Leu Val Ala Gln Trp	Gln Ala Thr Glu
2600	2605	2610
Pro Ala Val Pro Ala Glu	Pro Thr Ala Glu Pro	Pro Val Ala Asp
2615	2620	2625
Glu Asp Ala Ala Ala Ile	Ile Gly Leu Ala Gly	Arg Phe Pro Gly
2630	2635	2640
Ala Asp Thr Leu Glu Glu	Phe Trp Asn Asn Leu	Arg Asn Gly Gln
2645	2650	2655
Ser Ser Met Gly Glu Val	Pro Gly Glu Arg Trp	Asp His Gln His
2660	2665	2670
Tyr Phe Asp Ser Glu Arg	Gln Ala Pro Gly Lys	Thr Tyr Ser Arg
2675	2680	2685

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Leu	Asn	Pro	Tyr	Ile	Glu	Phe	Gly	Arg	Phe	Gln	Val	Gln	Gln	Gln
2990						2995					3000			
Pro	Ala	Pro	Trp	Pro	Arg	Arg	Gly	Ala	Gln	Pro	Arg	Arg	Ala	Gly
3005						3010					3015			
Leu	Ser	Ala	Phe	Gly	Ala	Gly	Gly	Ser	Asn	Ala	His	Leu	Val	Val
3020						3025					3030			
Glu	Glu	Ala	Pro	Ala	Met	Ala	Pro	Gly	Val	Ser	Ile	Ser	Ala	Ser
3035						3040					3045			
Ser	Pro	Ala	Leu	Ile	Val	Leu	Ser	Ala	Arg	Thr	Leu	Pro	Ala	Leu
3050						3055					3060			
Gln	Gln	Arg	Ala	Arg	Asp	Leu	Leu	Val	Trp	Met	Gln	Ala	Arg	Gln
3065						3070					3075			
Val	Asp	Asp	Val	Met	Leu	Ala	Asp	Val	Ala	Tyr	Thr	Leu	His	Leu
3080						3085					3090			
Gly	Arg	Val	Ala	Met	Glu	Gln	Arg	Leu	Ala	Phe	Thr	Ala	Gly	Ser
3095						3100					3105			
Ala	Ala	Glu	Leu	Ser	Glu	Lys	Leu	Gln	Ala	Tyr	Leu	Gly	His	Ala
3110						3115					3120			
Ile	Arg	Ala	Asp	Ile	Tyr	Leu	Ser	Glu	Asp	Thr	Pro	Gly	Lys	Pro
3125						3130					3135			
Ala	Gly	Ala	Pro	Ile	Val	Ala	Glu	Glu	Asp	Leu	Leu	Thr	Leu	Met
3140						3145					3150			
Asp	Ala	Trp	Ile	Glu	Lys	Gly	Gln	Tyr	Gly	Arg	Leu	Leu	Gly	Tyr
3155						3160					3165			
Trp	Thr	Lys	Gly	Gln	Pro	Ile	Asp	Trp	Asn	Lys	Leu	Tyr	Trp	Arg
3170						3175					3180			
Lys	Leu	Tyr	Ala	Asp	Gly	Arg	Pro	Arg	Arg	Ile	Ser	Leu	Pro	Thr
3185						3190					3195			
Tyr	Pro	Phe	Glu	His	Arg	Arg	Tyr	Trp	Gln	Thr	Pro	Val	Pro	Gly
3200						3205					3210			
Glu	Arg	Ser	Leu	His	Ala	Thr	Ala	Pro	Ala	Thr	Arg	Glu	Thr	Val
3215						3220					3225			
Ala	Val	Gly	Ala	Met	Pro	Asp	Pro	Ala	Gly	Ala	Thr	Val	Gln	Ala
3230						3235					3240			
Arg	Leu	Cys	Ala	Leu	Cys	Gln	Val	Leu	Leu	Gly	Lys	Pro	Val	Thr
3245						3250					3255			
Ala	Gln	Met	Asp	Phe	Phe	Ala	Val	Gly	Gly	His	Ser	Val	Leu	Ala
3260						3265					3270			
Ile	Gln	Leu	Val	Ser	Arg	Ile	Arg	Lys	Ser	Phe	Gly	Val	Glu	Tyr
3275						3280					3285			
Pro	Val	Ser	Ala	Leu	Phe	Glu	Ser	Ala	Leu	Leu	Ser	Asp	Met	Ala

3290						3295						3300							
Arg	Gln	Ile	Glu	Gln	Leu	Arg	Val	Asn	Gly	Val	Ala	Lys	Arg	Met					
3305						3310					3315								
Pro	Ala	Leu	Leu	Pro	Ala	Gly	Arg	Val	Gly	Ala	Ile	Pro	Ala	Thr					
3320						3325					3330								
Tyr	Ala	Gln	Glu	Arg	Leu	Trp	Leu	Val	His	Glu	His	Met	Ser	Glu					
3335						3340					3345								
Gln	Arg	Ser	Ser	Tyr	Asn	Ile	Thr	Phe	Ala	Met	His	Phe	Arg	Gly					
3350						3355					3360								
Val	Asp	Phe	Arg	Ala	Glu	Ala	Met	Arg	Ala	Ala	Leu	Asn	Ala	Leu					
3365						3370					3375								
Val	Val	Arg	His	Glu	Val	Leu	Arg	Thr	Arg	Phe	Leu	Ser	Glu	Asp					
3380						3385					3390								
Gly	Gln	Leu	Gln	Gln	Val	Ile	Ala	Ala	Ser	Leu	Thr	Leu	Glu	Val					
3395						3400					3405								
Pro	Val	Arg	Glu	Met	Ser	Val	Glu	Glu	Val	Asp	Leu	Leu	Leu	Ala					
3410						3415					3420								
Ala	Ser	Thr	Arg	Glu	Thr	Phe	Asp	Leu	Arg	Gln	Gly	Pro	Leu	Phe					
3425						3430					3435								
Lys	Ala	Arg	Ile	Leu	Arg	Val	Ala	Ala	Asp	His	His	Val	Val	Leu					
3440						3445					3450								
Ser	Ser	Ile	His	His	Ile	Ile	Ser	Asp	Gly	Trp	Ser	Leu	Gly	Val					
3455						3460					3465								
Phe	Asn	Arg	Asp	Leu	His	Gln	Leu	Tyr	Glu	Ala	Cys	Leu	Arg	Gly					
3470						3475					3480								
Thr	Pro	Pro	Thr	Leu	Pro	Thr	Leu	Ala	Val	Gln	Tyr	Ala	Asp	Tyr					
3485						3490					3495								
Ala	Leu	Trp	Gln	Arg	Gln	Trp	Glu	Leu	Ala	Ala	Pro	Leu	Ser	Tyr					
3500						3505					3510								
Trp	Thr	Arg	Ala	Leu	Glu	Gly	Tyr	Asp	Asp	Gly	Leu	Asp	Leu	Pro					
3515						3520					3525								
Tyr	Asp	Arg	Pro	Arg	Gly	Ala	Thr	Arg	Ala	Trp	Arg	Ala	Gly	Leu					
3530						3535					3540								
Val	Lys	His	Arg	Tyr	Pro	Pro	Gln	Leu	Ala	Gln	Gln	Leu	Ala	Ala					
3545						3550					3555								
Tyr	Ser	Gln	Gln	Tyr	Gln	Ala	Thr	Leu	Phe	Met	Ser	Leu	Leu	Ala					
3560						3565					3570								
Gly	Leu	Ala	Leu	Val	Leu	Gly	Arg	Tyr	Ala	Asp	Arg	Lys	Asp	Val					
3575						3580					3585								
Cys	Ile	Gly	Ala	Thr	Val	Ser	Gly	Arg	Asp	Gln	Leu	Glu	Leu	Glu					
3590						3595					3600								

Glu	Leu	Ile	Gly	Phe	Phe	Ile	Asn	Ile	Leu	Pro	Leu	Arg	Val	Asp
3605						3610					3615			
Leu	Ser	Gly	Asp	Pro	Cys	Leu	Glu	Glu	Val	Leu	Leu	Arg	Thr	Arg
3620						3625					3630			
Gln	Val	Val	Leu	Asp	Gly	Phe	Ala	His	Gln	Ser	Val	Pro	Phe	Glu
3635						3640					3645			
His	Val	Leu	Gln	Ala	Leu	Arg	Arg	Gln	Arg	Asp	Ser	Ser	Gln	Ile
3650						3655					3660			
Pro	Leu	Val	Pro	Val	Met	Leu	Arg	His	Gln	Asn	Phe	Pro	Thr	Gln
3665						3670					3675			
Glu	Ile	Gly	Asp	Trp	Pro	Glu	Gly	Val	Arg	Leu	Thr	Gln	Met	Glu
3680						3685					3690			
Leu	Gly	Leu	Asp	Arg	Ser	Thr	Pro	Ser	Glu	Leu	Asp	Trp	Gln	Phe
3695						3700					3705			
Tyr	Gly	Asp	Gly	Ser	Ser	Leu	Glu	Leu	Thr	Leu	Glu	Tyr	Ala	Gln
3710						3715					3720			
Asp	Leu	Phe	Asp	Glu	Ala	Thr	Val	Arg	Arg	Met	Ile	Ala	His	His
3725						3730					3735			
Gln	Gln	Ala	Leu	Glu	Ala	Met	Val	Ser	Arg	Pro	Gln	Leu	Arg	Val
3740						3745					3750			
Gly	Lys	Trp	Asp	Met	Leu	Thr	Ala	Glu	Glu	Arg	Arg	Leu	Phe	Ala
3755						3760					3765			
Ala	Leu	Asn	Ala	Thr	Gly	Thr	Pro	Arg	Glu	Trp	Pro	Ser	Leu	Ala
3770						3775					3780			
Gln	Gln	Phe	Glu	Arg	Gln	Ala	Gln	Ala	Thr	Pro	Gln	Ala	Ile	Ala
3785						3790					3795			
Cys	Val	Ser	Asp	Gly	Gln	Ser	Trp	Ser	Tyr	Ala	Gln	Leu	Glu	Ala
3800						3805					3810			
Arg	Ala	Asn	Gln	Leu	Ala	Gln	Ala	Leu	Arg	Gly	Gln	Gly	Ala	Gly
3815						3820					3825			
Arg	Asp	Val	Arg	Val	Ala	Val	Gln	Ser	Ala	Arg	Thr	Pro	Glu	Leu
3830						3835					3840			
Leu	Met	Ala	Leu	Leu	Ala	Ile	Phe	Lys	Ala	Gly	Ala	Cys	Tyr	Val
3845						3850					3855			
Pro	Ile	Asp	Pro	Ala	Tyr	Pro	Ala	Ala	Tyr	Arg	Glu	Gln	Ile	Leu
3860						3865					3870			
Ala	Glu	Val	Gln	Val	Ser	Ile	Val	Leu	Glu	Gln	Asp	Glu	Leu	Ala
3875						3880					3885			
Leu	Asp	Glu	Gln	Gly	Gln	Phe	His	Asn	Pro	Arg	Trp	Arg	Glu	Gln
3890						3895					3900			

Ala	Pro	Thr	Pro	Leu	Gly	Leu	Arg	Glu	His	Pro	Gly	Asp	Leu	Ala
3905						3910					3915			
Cys	Val	Met	Val	Thr	Ser	Gly	Ser	Thr	Gly	Arg	Pro	Lys	Gly	Val
3920						3925					3930			
Met	Val	Pro	Tyr	Ala	Gln	Leu	Tyr	Asn	Trp	Leu	His	Ala	Gly	Trp
3935						3940					3945			
Gln	Arg	Ser	Pro	Phe	Glu	Ala	Gly	Glu	Arg	Val	Leu	Gln	Lys	Thr
3950						3955					3960			
Ser	Ile	Ala	Phe	Ala	Val	Ser	Val	Lys	Glu	Leu	Leu	Ser	Gly	Leu
3965						3970					3975			
Leu	Ala	Gly	Val	Glu	Gln	Val	Met	Leu	Pro	Asp	Glu	Gln	Val	Lys
3980						3985					3990			
Asp	Ser	Leu	Ala	Leu	Ala	Arg	Ala	Ile	Glu	Gln	Trp	Gln	Val	Thr
3995						4000					4005			
Arg	Leu	Tyr	Leu	Val	Pro	Ser	His	Leu	Gln	Ala	Leu	Leu	Asp	Ala
4010						4015					4020			
Thr	Gln	Gly	Arg	Asp	Gly	Leu	Leu	His	Ser	Leu	Arg	His	Val	Val
4025						4030					4035			
Thr	Ala	Gly	Glu	Ala	Leu	Pro	Ser	Ala	Val	Arg	Glu	Thr	Val	Arg
4040						4045					4050			
Ala	Arg	Leu	Pro	Gln	Val	Gln	Leu	Trp	Asn	Asn	Tyr	Gly	Cys	Thr
4055						4060					4065			
Glu	Leu	Asn	Asp	Ala	Thr	Tyr	His	Arg	Ser	Asp	Thr	Val	Ala	Pro
4070						4075					4080			
Gly	Thr	Phe	Val	Pro	Ile	Gly	Ala	Pro	Ile	Ala	Asn	Thr	Glu	Val
4085						4090					4095			
Tyr	Val	Leu	Asp	Arg	Gln	Leu	Arg	Gln	Val	Pro	Ile	Gly	Val	Met
4100						4105					4110			
Gly	Glu	Leu	His	Val	His	Ser	Val	Gly	Met	Ala	Arg	Gly	Tyr	Trp
4115						4120					4125			
Asn	Arg	Pro	Gly	Leu	Thr	Ala	Ser	Arg	Phe	Ile	Ala	His	Pro	Tyr
4130						4135					4140			
Ser	Glu	Glu	Pro	Gly	Thr	Arg	Leu	Tyr	Lys	Thr	Gly	Asp	Met	Val
4145						4150					4155			
Arg	Arg	Leu	Ala	Asp	Gly	Thr	Leu	Glu	Tyr	Leu	Gly	Arg	Gln	Asp
4160						4165					4170			
Phe	Glu	Val	Lys	Val	Arg	Gly	His	Arg	Val	Asp	Thr	Arg	Gln	Val
4175						4180					4185			
Glu	Ala	Ala	Leu	Arg	Ala	Gln	Pro	Ala	Val	Ala	Glu	Ala	Val	Val
4190						4195					4200			
Ser	Gly	His	Arg	Val	Asp	Gly	Asp	Met	Gln	Leu	Val	Ala	Tyr	Val

4205		4210		4215
Val Ala Arg Glu Gly Gln	Ala Pro Ser Ala Gly	Glu Leu Lys Gln		
4220	4225	4230		
Gln Leu Ser Ala Gln Leu	Pro Thr Tyr Met Leu	Pro Thr Val Tyr		
4235	4240	4245		
Gln Trp Leu Glu Gln Leu	Pro Arg Leu Ser Asn	Gly Lys Leu Asp		
4250	4255	4260		
Arg Leu Ala Leu Pro Ala	Pro Gln Ala Val His	Ala Gln Glu Tyr		
4265	4270	4275		
Val Ala Pro Arg Asn Gln	Ala Glu Gln Arg Leu	Ala Ala Leu Phe		
4280	4285	4290		
Ala Glu Val Leu Arg Val	Glu Gln Val Gly Ile	His Asp Asn Phe		
4295	4300	4305		
Phe Ala Leu Gly Gly His	Ser Leu Ser Ala Ser	Gln Leu Ile Ser		
4310	4315	4320		
Arg Ile Ala Arg Asp Met	Ala Ile Asp Leu Pro	Leu Ala Met Leu		
4325	4330	4335		
Phe Glu Leu Pro Thr Val	Ala Gln Leu Ser Glu	Ser Leu Ala Ser		
4340	4345	4350		
His Ala Arg Asp Ser Asp	Tyr Asp Val Ile Pro	Ala Ser Thr Glu		
4355	4360	4365		
Glu Ala Thr Ile Pro Leu	Ser Thr Ala Gln Glu	Arg Met Trp Phe		
4370	4375	4380		
Leu His Lys Phe Val Gln	Glu Thr Pro Tyr Asn	Thr Pro Gly Leu		
4385	4390	4395		
Ala Leu Leu Gln Gly Glu	Leu Asp Ile Ser Ala	Leu Gln Val Ala		
4400	4405	4410		
Phe Arg Cys Val Leu Glu	Arg His Ala Val Leu	Arg Thr His Phe		
4415	4420	4425		
Val Glu Thr Glu Gln Gln	Cys Val Gln Val Ile	Gly Ala Ala Glu		
4430	4435	4440		
Gln Phe Val Leu Gln Leu	Arg Ser Ile Arg Asp	Glu Ala Asp Leu		
4445	4450	4455		
His Gly Leu Leu His Thr	Ala Val Ser Glu Pro	Phe Asp Leu Glu		
4460	4465	4470		
Arg Glu Leu Pro Leu Arg	Ala Leu Leu Tyr Arg	Leu Asp Asp Arg		
4475	4480	4485		
Arg His Tyr Leu Ala Val	Val Ile His His Ile	Val Phe Asp Gly		
4490	4495	4500		
Trp Ser Thr Ser Ile Leu	Phe Arg Glu Leu Ala	Thr His Tyr Ala		
4505	4510	4515		

Ala Cys Arg His Gly Gln Ser Ala Pro Leu Pro Pro Leu Glu Leu	4520	4525	4530
Ser Tyr Ala Asp Tyr Ala Arg Trp Glu Arg Ala Arg Leu Asn Gln	4535	4540	4545
Glu Asp Ala Leu Arg Lys Leu Glu Tyr Trp Lys Thr Gln Leu Ala	4550	4555	4560
Asp Ala Pro Pro Leu Val Leu Pro Thr Thr Tyr Ala Arg Pro Val	4565	4570	4575
Phe Gln Asn Phe Asn Gly Ala Thr Val Ala Leu Gln Ile Glu Pro	4580	4585	4590
Pro Leu Leu Gln Arg Leu Gln Arg Phe Ala Asp Ala His Ser Phe	4595	4600	4605
Thr Leu Tyr Met Leu Leu Leu Ala Ala Leu Gly Val Val Leu Ser	4610	4615	4620
Arg His Ala Arg Gln Lys His Phe Cys Ile Gly Ser Pro Val Ala	4625	4630	4635
Asn Arg Ala Arg Ala Glu Leu His Gly Leu Ile Gly Leu Phe Val	4640	4645	4650
Asn Thr Leu Ala Val Arg Leu Asp Leu Asp Gly Asn Pro Ser Val	4655	4660	4665
Arg Glu Leu Leu Glu Arg Ile His Cys Thr Thr Leu Ala Ala Tyr	4670	4675	4680
Glu His Gln Asp Val Pro Phe Glu Arg Ile Val Glu Ser Leu Lys	4685	4690	4695
Val Pro Arg Asp Thr Ala Arg Asn Pro Leu Gly Gln Val Met Leu	4700	4705	4710
Asn Phe Gln Asn Met Pro Met Ser Ala Phe Asp Leu Asp Gly Val	4715	4720	4725
Gln Val Gln Val Leu Pro Met His Asn Gly Thr Ala Lys Cys Glu	4730	4735	4740
Leu Thr Phe Asp Leu Leu Leu Asp Gly Ser Arg Leu Ser Gly Phe	4745	4750	4755
Val Glu Tyr Ala Thr Gly Leu Phe Ala Pro Glu Trp Val Gln Ala	4760	4765	4770
Leu Val Gln Gln Phe Lys Cys Val Leu Ala Ala Leu Val Glu Arg	4775	4780	4785
Pro Glu Ala Ser Leu Asn Asp Leu Pro Met Ala Pro Asn Glu Ala	4790	4795	4800
Gln Pro Ala Ser Pro Ala Leu Met Lys His Val Ala Pro Ser Leu	4805	4810	4815
Pro Asn Leu Leu Glu Ala Met Ala Ala Asn Asp Ala Ala Arg Leu			

4820	4825	4830
Ala Leu Gln Ala Pro Glu Gly	Ala Leu Ser Tyr Ala	Gln Leu Ile
4835	4840	4845
Glu Ala Ala Asn Glu Phe Ala	Trp Arg Leu Arg Cys	Glu His Ala
4850	4855	4860
Gly Pro Asp Lys Val Val Ala	Leu Cys Leu Ala Pro	Cys Ser Ala
4865	4870	4875
Leu Val Val Ala Leu Leu Ala	Ala Ser Leu Cys Gly	Ala Ala Ser
4880	4885	4890
Val Leu Ile Asp Pro Thr Thr	Thr Ala Glu Ala Gln	Tyr Asp Gln
4895	4900	4905
Leu Phe Glu Thr Arg Ala Gly	Ile Val Val Thr Cys	Ser Ser Leu
4910	4915	4920
Leu Glu Lys Leu Pro Leu Asp	Asp Gln Ala Val Val	Leu Ile Asp
4925	4930	4935
Glu Gln Ala Ala Glu Ala Thr	Pro Arg Leu Met His	Phe Thr Asp
4940	4945	4950
Asp Pro Ala Leu Pro Ala Met	Leu Tyr Cys Val Cys	Asp Glu Lys
4955	4960	4965
Gly Arg Thr Arg Thr Ile Met	Val Glu Ser Gly Ser	Leu Ser Ser
4970	4975	4980
Arg Leu Leu Asp Ser Val Gln	Arg Phe Ser Leu Glu	Arg Thr Asp
4985	4990	4995
Arg Phe Leu Leu Arg Ser Pro	Leu Ser Ala Glu Leu	Ala Asn Thr
5000	5005	5010
Glu Val Leu Gln Trp Leu Ala	Ala Gly Gly Ser Leu	Ser Ile Ala
5015	5020	5025
Pro Met His Gly Asp Phe Asp	Ala Ala Ala Trp Leu	Glu Thr Leu
5030	5035	5040
Ala Thr Tyr Ala Ile Thr Val	Ala Tyr Leu Ala Gln	Val Glu Leu
5045	5050	5055
Thr Glu Met Leu Ala His Leu	Gln Asn His Pro Leu	Glu Arg Asn
5060	5065	5070
Lys Leu Ala Gly Leu Arg Val	Leu Val Val His Gly	Ala Pro Leu
5075	5080	5085
Pro Ile Ala Pro Leu Met Arg	Leu Asp Ala Trp Leu	Arg Glu Val
5090	5095	5100
Gly Gly Ser Ala Arg Ile Phe	Ala Ala Tyr Gly Asn	Ala Glu Phe
5105	5110	5115
Gly Ala Glu Ile Leu Ser Gln	Asp Val Ser Ala Ala	Leu Gln Ala
5120	5125	5130

Gly Ile 5135	Gly Ala Gln Tyr 5140	Lys His Arg Arg Gly Leu 5145	Phe Pro Leu
Gly Ala 5150	Asn Ser Met Cys 5155	His Val Val Gln Ser 5160	Gly Arg Ile
Ala Pro 5165	Asp Gly Met Val 5170	Gly Glu Leu Trp Ile 5175	Thr Gln Pro Ala
Cys Leu 5180	Tyr Lys Thr Asp 5185	Ala Leu Val Arg Arg 5190	Leu Ala Asn Gly
Gln Leu 5195	Glu Trp Leu Gly 5200	Ser Leu Asp Val Gln 5205	Ser Arg Ile Asp
Asp Pro 5210	Arg Ile Asp Leu 5215	Cys Val Val Glu Ala 5220	Gln Leu Arg Leu
Cys Glu 5225	Asp Val Gly Glu 5230	Ala Val Val Leu Tyr 5235	Glu Pro Leu Lys
Arg Cys 5240	Leu Val Ala Tyr 5245	Leu Ser Ala Arg Ser 5250	Thr Ala Ala Ile
Met Thr 5255	Asp Glu Thr Leu 5260	Ala Arg Ile Arg Gln 5265	Ala Leu Ser Glu
Thr Leu 5270	Pro Asp Tyr Leu 5275	Leu Pro Ala Ile Trp 5280	Val Pro Leu Ala
His Trp 5285	Pro Arg Leu Pro 5290	His Gly Arg Val Asp 5295	Leu Gly Ala Leu
Pro Ala 5300	Pro Asp Phe Asp 5305	Leu Ala Arg His Glu 5310	Ser Tyr Ile Ala
Pro Arg 5315	Thr Ala Val Glu 5320	Gln Ala Val Ala Glu 5325	Ile Trp Gln Arg
Val Leu 5330	Lys Arg Thr Gln 5335	Val Gly Val His Asp 5340	Asn Phe Phe Glu
Leu Gly 5345	Gly His Ser Val 5350	Leu Ala Ile Gln Leu 5355	Val Ser Gly Leu
Arg Lys 5360	Ala Leu Ala Ile 5365	Glu Val Pro Val Thr 5370	Leu Val Phe Glu
Ala Pro 5375	Ile Leu Gly Ala 5380	Leu Ala Arg Gln Ile 5385	Ala Pro Leu Leu
Val Ser 5390	Glu Arg Arg Pro 5395	Arg Pro Pro Gly Leu 5400	Thr Arg Leu Glu
His Thr 5405	Gly Pro Ile Pro 5410	Ala Ser Tyr Ala Gln 5415	Glu Arg Leu Trp
Leu Val 5420	His Glu His Met 5425	Glu Glu Gln Arg Thr 5430	Ser Tyr Asn Ile

Ser	Asn	Ala	Ala	His	Phe	Ile	Gly	Ala	Ala	Phe	Ser	Val	Glu	Ala
5435						5440					5445			
Met	Arg	Ala	Ala	Leu	Asn	Ala	Leu	Val	Ala	Arg	His	Glu	Val	Leu
5450						5455					5460			
Arg	Thr	Arg	Phe	Leu	Ser	Glu	Asp	Gly	Gln	Leu	Gln	Gln	Val	Ile
5465						5470					5475			
Ala	Ala	Ser	Leu	Thr	Leu	Glu	Val	Pro	Val	Arg	Glu	Val	Ser	Ala
5480						5485					5490			
Glu	Glu	Val	Asp	Leu	Leu	Leu	Ala	Ala	Ser	Thr	Arg	Glu	Thr	Phe
5495						5500					5505			
Asp	Leu	Arg	Gln	Gly	Pro	Leu	Phe	Lys	Ala	Arg	Ile	Leu	Arg	Val
5510						5515					5520			
Ala	Ala	Asp	His	His	Val	Val	Leu	Ser	Ser	Ile	His	His	Ile	Ile
5525						5530					5535			
Ser	Asp	Gly	Trp	Ser	Leu	Gly	Val	Phe	Asn	Arg	Asp	Leu	His	Gln
5540						5545					5550			
Leu	Tyr	Glu	Ala	Cys	Leu	Arg	Gly	Thr	Pro	Pro	Thr	Leu	Pro	Thr
5555						5560					5565			
Leu	Ala	Val	Gln	Tyr	Ala	Asp	Tyr	Ala	Leu	Trp	Gln	Arg	Gln	Trp
5570						5575					5580			
Glu	Leu	Ala	Ala	Pro	Leu	Ser	Tyr	Trp	Thr	Arg	Ala	Leu	Glu	Gly
5585						5590					5595			
Tyr	Asp	Asp	Gly	Leu	Asp	Leu	Pro	Tyr	Asp	Arg	Pro	Arg	Gly	Ala
5600						5605					5610			
Thr	Arg	Ala	Trp	Arg	Ala	Gly	Leu	Val	Lys	His	Arg	Tyr	Pro	Pro
5615						5620					5625			
Gln	Leu	Ala	Gln	Gln	Leu	Ala	Ala	Tyr	Ser	Gln	Gln	Tyr	Gln	Ala
5630						5635					5640			
Thr	Leu	Phe	Met	Ser	Leu	Leu	Ala	Gly	Leu	Ala	Leu	Val	Leu	Gly
5645						5650					5655			
Arg	Tyr	Ala	Asp	Arg	Lys	Asp	Val	Cys	Ile	Gly	Ala	Thr	Val	Ser
5660						5665					5670			
Gly	Arg	Asp	Gln	Leu	Glu	Leu	Glu	Glu	Leu	Ile	Gly	Phe	Phe	Ile
5675						5680					5685			
Asn	Ile	Leu	Pro	Leu	Arg	Val	Asp	Leu	Ser	Gly	Asp	Pro	Cys	Leu
5690						5695					5700			
Glu	Glu	Val	Leu	Leu	Arg	Thr	Arg	Gln	Val	Val	Leu	Asp	Gly	Phe
5705						5710					5715			
Ala	His	Gln	Ser	Val	Pro	Phe	Glu	His	Val	Leu	Gln	Ala	Leu	Arg
5720						5725					5730			
Arg	Gln	Arg	Asp	Ser	Ser	Gln	Ile	Pro	Leu	Val	Pro	Val	Met	Leu

5735					5740					5745				
Arg	His	Gln	Asn	Phe	Pro	Thr	Gln	Glu	Ile	Gly	Asp	Trp	Pro	Glu
5750						5755					5760			
Gly	Val	Arg	Leu	Thr	Gln	Met	Glu	Leu	Gly	Leu	Asp	Arg	Ser	Thr
5765						5770					5775			
Pro	Ser	Glu	Leu	Asp	Trp	Gln	Phe	Tyr	Gly	Asp	Gly	Ser	Ser	Leu
5780						5785					5790			
Glu	Leu	Thr	Leu	Glu	Tyr	Ala	Gln	Asp	Leu	Phe	Asp	Glu	Ala	Thr
5795						5800					5805			
Val	Arg	Arg	Met	Ile	Ala	His	His	Gln	Gln	Ala	Leu	Glu	Ala	Met
5810						5815					5820			
Val	Ser	Arg	Pro	Gln	Leu	Arg	Val	Gly	Lys	Trp	Asp	Met	Leu	Thr
5825						5830					5835			
Ala	Glu	Glu	Arg	Arg	Leu	Phe	Ala	Ala	Leu	Asn	Ala	Thr	Gly	Thr
5840						5845					5850			
Pro	Arg	Glu	Trp	Pro	Ser	Leu	Ala	Gln	Gln	Phe	Glu	Arg	Gln	Ala
5855						5860					5865			
Gln	Ala	Thr	Pro	Gln	Ala	Ile	Ala	Cys	Val	Ser	Asp	Gly	Gln	Ser
5870						5875					5880			
Trp	Ser	Tyr	Ala	Gln	Leu	Glu	Ala	Arg	Ala	Asn	Gln	Leu	Ala	Gln
5885						5890					5895			
Ala	Leu	Arg	Gly	Gln	Gly	Ala	Gly	Arg	Asp	Val	Arg	Val	Ala	Val
5900						5905					5910			
Gln	Ser	Ala	Arg	Thr	Pro	Glu	Leu	Leu	Met	Ala	Leu	Leu	Ala	Ile
5915						5920					5925			
Phe	Lys	Ala	Gly	Ala	Cys	Tyr	Val	Pro	Ile	Asp	Pro	Ala	Tyr	Pro
5930						5935					5940			
Ala	Ala	Tyr	Arg	Glu	Gln	Ile	Leu	Ala	Glu	Val	Gln	Val	Ser	Ile
5945						5950					5955			
Val	Leu	Glu	Gln	Gly	Glu	Leu	Ala	Leu	Asp	Glu	Gln	Gly	Gln	Phe
5960						5965					5970			
Arg	Asn	Arg	Arg	Trp	Arg	Glu	Gln	Ala	Pro	Thr	Pro	Leu	Gly	Leu
5975						5980					5985			
Arg	Gly	His	Pro	Gly	Asp	Leu	Ala	Cys	Val	Met	Val	Thr	Ser	Gly
5990						5995					6000			
Ser	Thr	Gly	Arg	Pro	Lys	Gly	Val	Met	Val	Pro	Tyr	Ala	Gln	Leu
6005						6010					6015			
His	Asn	Trp	Leu	His	Ala	Gly	Trp	Gln	Arg	Ser	Ala	Phe	Glu	Ala
6020						6025					6030			
Gly	Glu	Arg	Val	Leu	Gln	Lys	Thr	Ser	Ile	Ala	Phe	Ala	Val	Ser
6035						6040					6045			

Val	Lys	Glu	Leu	Leu	Ser	Gly	Leu	Leu	Ala	Gly	Val	Gly	Gln	Val
6050						6055					6060			
Met	Leu	Pro	Asp	Glu	Gln	Val	Lys	Asp	Ser	Leu	Ala	Leu	Ala	Arg
6065						6070					6075			
Ala	Ile	Glu	Gln	Trp	Gln	Val	Thr	Arg	Leu	Tyr	Leu	Val	Pro	Ser
6080						6085					6090			
His	Leu	Gln	Ala	Leu	Leu	Asp	Ala	Thr	Gln	Gly	Arg	Asp	Gly	Leu
6095						6100					6105			
Leu	His	Ser	Leu	Arg	His	Val	Val	Thr	Ala	Gly	Glu	Ala	Leu	Pro
6110						6115					6120			
Ser	Ala	Val	Gly	Glu	Ala	Val	Arg	Val	Arg	Leu	Pro	Gln	Val	Gln
6125						6130					6135			
Leu	Trp	Asn	Asn	Tyr	Gly	Cys	Thr	Glu	Leu	Asn	Asp	Ala	Thr	Tyr
6140						6145					6150			
His	Arg	Ser	Asp	Thr	Val	Ala	Pro	Gly	Thr	Phe	Val	Pro	Ile	Gly
6155						6160					6165			
Ala	Pro	Ile	Ala	Asn	Thr	Glu	Val	Tyr	Val	Leu	Asp	Arg	Gln	Leu
6170						6175					6180			
Arg	Gln	Val	Pro	Ile	Gly	Val	Met	Gly	Glu	Leu	His	Val	His	Ser
6185						6190					6195			
Val	Gly	Met	Ala	Arg	Gly	Tyr	Trp	Asn	Arg	Pro	Gly	Leu	Thr	Ala
6200						6205					6210			
Ser	Arg	Phe	Ile	Ala	His	Pro	Tyr	Ser	Glu	Glu	Pro	Gly	Thr	Arg
6215						6220					6225			
Leu	Tyr	Lys	Thr	Gly	Asp	Met	Val	Arg	Arg	Leu	Ala	Asp	Gly	Thr
6230						6235					6240			
Leu	Glu	Tyr	Leu	Gly	Arg	Gln	Asp	Phe	Glu	Val	Lys	Val	Arg	Gly
6245						6250					6255			
His	Arg	Val	Asp	Thr	Arg	Gln	Val	Glu	Ala	Ala	Leu	Arg	Ala	Gln
6260						6265					6270			
Pro	Ala	Val	Ala	Glu	Ala	Val	Val	Ser	Gly	His	Arg	Val	Asp	Gly
6275						6280					6285			
Asp	Met	Gln	Leu	Val	Ala	Tyr	Val	Val	Ala	Arg	Glu	Gly	Gln	Ala
6290						6295					6300			
Pro	Ser	Ala	Gly	Glu	Leu	Lys	Gln	Gln	Leu	Ser	Ala	Gln	Leu	Pro
6305						6310					6315			
Thr	Tyr	Met	Leu	Pro	Thr	Val	Tyr	Gln	Trp	Leu	Glu	Gln	Leu	Pro
6320						6325					6330			
Arg	Leu	Ser	Asn	Gly	Lys	Leu	Asp	Arg	Leu	Ala	Leu	Pro	Ala	Pro
6335						6340					6345			
Gln	Val	Val	His	Ala	Gln	Glu	Tyr	Val	Ala	Pro	Arg	Asn	Glu	Ala

6350		6355		6360
Glu Gln Arg Leu Ala Ala	Leu Phe Ala Glu Val	Leu Arg Val Glu		
6365	6370	6375		
Gln Val Gly Ile His Asp	Asn Phe Phe Ala Leu	Gly Gly His Ser		
6380	6385	6390		
Leu Ser Ala Ser Gln Leu	Ile Ser Arg Ile Arg	Gln Ser Phe His		
6395	6400	6405		
Val Asp Leu Pro Leu Ser	Arg Ile Phe Glu Ala	Pro Thr Ile Glu		
6410	6415	6420		
Gly Leu Val Arg Gln Leu	Ala Leu Pro Ser Glu	Gly Gly Val Ala		
6425	6430	6435		
Ser Ile Ala Arg Val Ala	Arg Asn Arg Thr Ile	Pro Leu Ser Leu		
6440	6445	6450		
Phe Gln Glu Arg Leu Trp	Phe Val His Gln His	Met Pro Glu Gln		
6455	6460	6465		
Arg Thr Ser Tyr Asn Gly	Thr Leu Ala Leu Arg	Leu Arg Gly Pro		
6470	6475	6480		
Leu Ser Val Glu Ala Met	Arg Ala Ala Leu Arg	Ala Leu Val Leu		
6485	6490	6495		
Arg His Glu Ile Leu Arg	Thr Arg Phe Val Leu	Pro Thr Gly Ala		
6500	6505	6510		
Ser Glu Pro Val Gln Val	Ile Asp Glu His Ser	Asp Phe Gln Leu		
6515	6520	6525		
Ser Val Gln Leu Val Glu	Asp Thr Glu Ile Ala	Ser Leu Met Asp		
6530	6535	6540		
Glu Leu Ala Ser His Ile	Tyr Asp Leu Ala Asn	Gly Pro Leu Phe		
6545	6550	6555		
Ile Ala Cys Leu Leu Gln	Leu Asp Glu Gln Glu	His Val Leu Leu		
6560	6565	6570		
Ile Gly Met His His Leu	Ile Tyr Asp Ala Trp	Ser Gln Phe Thr		
6575	6580	6585		
Val Met Asn Arg Asp Leu	Arg Val Leu Tyr His	Arg His Leu Gly		
6590	6595	6600		
Leu Ala Gly Gly Asp Leu	Pro Glu Leu Pro Ile	Gln Tyr Ala Asp		
6605	6610	6615		
Tyr Ala Ile Trp Gln Arg	Ala Gln Asn Leu Asp	Ala Gln Leu Ala		
6620	6625	6630		
Tyr Trp Gln Ala Met Leu	His Asp Tyr Asp Asp	Gly Leu Glu Leu		
6635	6640	6645		
Pro Tyr Asp Tyr Pro Arg	Pro Arg Asn Arg Thr	Trp His Ala Ala		
6650	6655	6660		

Val Tyr Thr His Thr Tyr Pro Ala Glu Leu Val Gln Arg Phe Ala
 6665 6670 6675
 Gly Phe Val Gln Ala His Gln Ser Thr Leu Phe Ile Gly Leu Leu
 6680 6685 6690
 Ala Ser Phe Ala Val Val Leu Asn Lys Tyr Thr Gly Arg Asp Asp
 6695 6700 6705
 Leu Cys Ile Gly Thr Thr Thr Ala Gly Arg Thr His Leu Glu Leu
 6710 6715 6720
 Glu Asn Leu Ile Gly Phe Phe Ile Asn Ile Leu Pro Leu Arg Leu
 6725 6730 6735
 Arg Leu Asp Gly Asp Pro Asp Val Ala Glu Ile Met Arg Arg Thr
 6740 6745 6750
 Arg Leu Val Ala Met Ser Ala Phe Glu Asn Gln Ala Leu Pro Phe
 6755 6760 6765
 Glu His Leu Leu Asn Ala Leu His Lys Gln Arg Asp Thr Ser Arg
 6770 6775 6780
 Ile Pro Leu Val Pro Val Val Met Arg His Gln Asn Phe Pro Asp
 6785 6790 6795
 Thr Ile Gly Asp Trp Ser Asp Gly Ile Arg Thr Glu Val Ile Gln
 6800 6805 6810
 Arg Asp Leu Arg Ala Thr Pro Asn Glu Met Asp Leu Gln Phe Phe
 6815 6820 6825
 Gly Asp Gly Thr Gly Leu Ser Val Thr Val Glu Tyr Ala Ala Glu
 6830 6835 6840
 Leu Phe Ser Glu Ala Thr Ile Arg Arg Leu Ile His His His Gln
 6845 6850 6855
 Leu Val Leu Glu Gln Met Leu Ala Ala His Glu Ser Ala Thr Cys
 6860 6865 6870
 Pro Leu Asp Val Ala Asp
 6875

<210> 27
 <211> 343
 <212> PRT
 <213> Xanthomonas albilineans

<400> 27

Met Asp Ser Ala Leu Pro Thr Ser Ala Phe Thr Phe Asp Leu Phe Tyr
 1 5 10 15
 Thr Thr Val Asn Ala Tyr Tyr Arg Thr Ala Ala Val Lys Ala Ala Ile
 20 25 30
 Glu Leu Gly Leu Phe Asp Val Val Gly Gln Gln Gly Arg Thr Pro Ala
 35 40 45

Ala Ile Ala Glu Ala Cys Gln Ala Ser Pro Arg Gly Ile Arg Ile Leu
 50 55 60
 Cys Tyr Tyr Leu Val Ser Ile Gly Phe Leu Arg Arg Asn Gly Gly Leu
 65 70 75 80
 Phe Tyr Ile Asp Arg Asn Met Ala Met Tyr Leu Asp Arg Ser Ser Pro
 85 90 95
 Gly Tyr Leu Gly Gly Ser Ile Lys Phe Leu Leu Ser Pro Tyr Ile Met
 100 105 110
 Ser Ala Phe Thr Asp Leu Thr Ala Val Val Arg Thr Gly Lys Ile Asn
 115 120 125
 Leu Ala Gln Asp Gly Val Val Ala Pro Asp His Pro Gln Trp Val Glu
 130 135 140
 Phe Ala Arg Ala Met Ala Pro Met Met Ala Leu Pro Ser Ala Leu Ile
 145 150 155 160
 Ala Asn Met Val Ser Leu Pro Ala Asp Arg Pro Ile Arg Val Leu Asp
 165 170 175
 Val Ala Ala Gly His Gly Leu Phe Gly Ile Ala Phe Ala Gln Arg Phe
 180 185 190
 Arg Gln Ala Glu Val Ser Phe Leu Asp Trp Asp Asn Val Leu Asp Val
 195 200 205
 Ala Arg Glu Asn Ala Gln Ala Ala Lys Val Ala Glu Arg Ala Arg Phe
 210 215 220
 Leu Pro Gly Asn Ala Phe Asp Leu Asp Tyr Gly Ser Gly Tyr Asp Val
 225 230 235 240
 Ile Leu Leu Thr Asn Phe Leu His His Phe Asp Glu Val Asp Gly Glu
 245 250 255
 Arg Ile Leu Ala Lys Thr Arg Asp Ala Leu Asn Asp Asp Gly Met Val
 260 265 270
 Ile Thr Phe Glu Phe Ile Ala Asp Glu Glu Arg Ser Ser Pro Pro Leu
 275 280 285
 Ala Ala Thr Phe Ser Met Met Met Leu Gly Thr Thr Pro Ala Gly Glu
 290 295 300
 Ser Tyr Thr Tyr Ser Asp Leu Glu Arg Met Phe Arg His Ala Gly Phe
 305 310 315 320
 Gly His Val Glu Leu Lys Ser Ile Pro Pro Ala Leu Leu Lys Val Val
 325 330 335
 Val Ser Arg Lys Arg Ala Pro
 340

<210> 28

<211> 167

<212> PRT

<213> Xanthomonas albilineans

<400> 28

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Met Ile Glu Ser Ala Thr Ser Pro Val Ala Lys Thr Glu Arg Ile Trp
1           5           10           15

Cys Thr Glu Leu Asp Leu Asp Ala Leu Asn Ala Met Ser Ala Asn Thr
          20           25           30

Met Gln Ala Leu Leu Gly Ile Arg Met Ile Glu Ile Gly Ser Asp Tyr
          35           40           45

Leu Val Ser Cys Met Ser Val Asp Trp Arg Cys His Gln Pro Tyr Gly
          50           55           60

Val Leu His Gly Gly Ala Ser Val Thr Leu Ala Glu Ala Thr Gly Ser
65           70           75           80

Met Ala Ala Ser Met Cys Val Pro Ala Gly Gln Arg Cys Val Gly Leu
          85           90           95

Asp Ile Asn Ala Asn His Ile Ala Ser Ile Ser Ser Gly Gln Val Gln
          100          105          110

Cys Ile Ala Arg Pro Leu His Ile Gly Ala Leu Thr Gln Val Trp Gln
          115          120          125

Met Arg Ile Tyr Asp Glu Gly Asp Arg Thr Ile Cys Val Ser Arg Leu
          130          135          140

Thr Met Ala Val Leu Ser Val His Val Ala Arg Val Ser Pro Asn Pro
145          150          155          160

Ala Ser Ser Gly Val Gln Thr
          165

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<210> 29

<211> 941

<212> PRT

<213> Xanthomonas albilineans

<400> 29

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Met Asn Glu Thr Ala Thr Val Thr Lys Ala Thr Leu Ser Ser Ala Lys
1           5           10           15

Ala Ser Ile Thr Pro Ala Cys Val His Gln Trp Phe Glu Ala Gln Val
          20           25           30

Ser Ser Thr Pro Asp Ala Pro Ala Ala Phe Leu Gly Glu Arg Arg Met
          35           40           45

Ser Tyr Gly Gln Leu Asn Thr Arg Ala Asn Arg Leu Ala Arg Leu Leu
          50           55           60

Gln Ser Gln Gly Val Gly Pro Gly Ala Arg Val Ala Val Trp Met Asn
65           70           75           80

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Arg	Ser	Pro	Glu	Cys	Leu	Ala	Ala	Leu	Leu	Ala	Val	Met	Lys	Ala	Gly
				85					90					95	
Ala	Ala	Tyr	Val	Pro	Ile	Asp	Leu	Ser	Leu	Pro	Ile	Arg	Arg	Val	Gln
			100					105					110		
Tyr	Ile	Leu	Gln	Asp	Ser	Gln	Ala	Arg	Leu	Val	Leu	Val	Asp	Asp	Glu
		115					120					125			
Gly	Gln	Gly	Arg	Leu	Asp	Glu	Leu	Glu	Leu	Gly	Ala	Met	Thr	Ala	Val
	130					135					140				
Asp	Val	Cys	Gly	Thr	Leu	Asp	Gly	Asp	Glu	Ala	Asn	Leu	Asp	Leu	Pro
145					150					155					160
Cys	Asp	Pro	Ala	Gln	Pro	Val	Tyr	Cys	Ile	Tyr	Thr	Ser	Gly	Ser	Thr
				165					170					175	
Gly	Ser	Pro	Lys	Gly	Val	Leu	Val	Arg	His	Ser	Gly	Leu	Ala	Asn	Tyr
			180					185					190		
Val	Ala	Trp	Ala	Lys	Arg	Gln	Tyr	Val	Thr	Ala	Asp	Thr	Thr	Ser	Phe
		195					200					205			
Ala	Phe	Tyr	Ser	Ser	Leu	Ser	Phe	Asp	Leu	Thr	Val	Thr	Ser	Ile	Tyr
	210					215					220				
Val	Pro	Leu	Val	Ala	Gly	Leu	Cys	Val	His	Val	Tyr	Pro	Glu	Gln	Gly
225					230					235					240
Asp	Asp	Val	Pro	Val	Ile	Asn	Arg	Val	Leu	Asp	Asp	Asn	Gln	Val	Asp
				245					250				255		
Val	Ile	Lys	Leu	Thr	Pro	Ser	His	Met	Leu	Met	Leu	Arg	Asn	Ala	Ala
		260						265					270		
Leu	Ala	Thr	Ser	Arg	Leu	Lys	Thr	Leu	Ile	Val	Gly	Gly	Glu	Asp	Leu
		275					280					285			
Lys	Ala	Ala	Val	Ala	Tyr	Asp	Ile	His	Gln	Arg	Phe	Arg	Arg	Asp	Val
	290					295					300				
Ala	Ile	Tyr	Asn	Glu	Tyr	Gly	Pro	Thr	Glu	Thr	Val	Val	Gly	Cys	Ala
305					310					315					320
Ile	His	Arg	Tyr	Asp	Pro	Ala	Thr	Glu	Arg	Glu	Gly	Ser	Val	Pro	Ile
			325						330					335	
Gly	Val	Pro	Ile	Asp	His	Thr	Ser	Leu	His	Leu	Leu	Asp	Glu	Arg	Leu
			340					345					350		
Gln	Pro	Val	Ala	Pro	Gly	Glu	Val	Gly	Gln	Ile	His	Ile	Gly	Gly	Ala
		355					360					365			
Gly	Val	Ala	Ile	Gly	Tyr	Val	Asn	Lys	Pro	Glu	Ile	Thr	Asp	Ala	Gln
	370					375					380				
Phe	Ile	Asp	Asn	Pro	Phe	Glu	Gly	Ser	Gly	Arg	Leu	Tyr	Ala	Ser	Gly
385					390					395					400
Asp	Leu	Gly	Arg	Met	Arg	Ala	Asp	Gly	Lys	Leu	Glu	Phe	Leu	Gly	Arg

405										410					415				
Lys	Asp	Ser	Gln	Ile	Lys	Leu	Arg	Gly	Tyr	Arg	Ile	Glu	Leu	Gly	Glu				
			420					425					430						
Ile	Glu	Asn	Val	Leu	Leu	Gly	His	Ala	Ala	Leu	Arg	Glu	Cys	Ile	Val				
		435					440					445							
Asp	Thr	Thr	Val	Ala	Pro	Arg	Arg	Asp	Tyr	Asp	Ser	Lys	Ser	Leu	Arg				
	450					455					460								
Tyr	Cys	Ala	Arg	Cys	Gly	Ile	Ala	Ser	Asn	Phe	Pro	Asn	Thr	Ser	Phe				
465					470					475					480				
Asp	Glu	His	Gly	Val	Cys	Asn	His	Cys	His	Ala	Tyr	Asp	Lys	Tyr	Arg				
				485					490					495					
Asn	Val	Val	Glu	Asp	Tyr	Phe	Arg	Thr	Glu	Asp	Glu	Leu	Arg	Thr	Ile				
			500					505					510						
Phe	Glu	Gln	Val	Lys	Ala	His	Asn	Arg	Leu	Arg	Tyr	Asp	Cys	Leu	Val				
		515					520					525							
Ala	Phe	Ser	Gly	Gly	Lys	Asp	Ser	Thr	Tyr	Ala	Leu	Cys	Arg	Val	Val				
	530					535					540								
Asp	Met	Gly	Leu	Arg	Val	Leu	Ala	Tyr	Thr	Leu	Asp	Asn	Gly	Tyr	Ile				
545					550					555					560				
Ser	Asp	Glu	Ala	Lys	Ala	Asn	Val	Asp	Arg	Val	Val	Arg	Glu	Leu	Gly				
				565					570					575					
Val	Asp	His	Arg	Tyr	Leu	Gly	Thr	Pro	His	Met	Asn	Ala	Ile	Phe	Val				
			580					585					590						
Asp	Ser	Leu	His	Arg	His	Ser	Asn	Val	Cys	Asn	Gly	Cys	Phe	Lys	Thr				
		595					600					605							
Ile	Tyr	Thr	Leu	Gly	Ile	Asn	Leu	Ala	His	Glu	Val	Gly	Val	Ser	Asp				
	610					615					620								
Ile	Val	Met	Gly	Leu	Ser	Lys	Gly	Gln	Leu	Phe	Glu	Thr	Arg	Leu	Ser				
625					630					635					640				
Glu	Leu	Phe	Arg	Ala	Ser	Thr	Phe	Asp	Asn	Gln	Val	Phe	Glu	Lys	Asn				
				645					650					655					
Leu	Met	Glu	Ala	Arg	Lys	Ile	Tyr	His	Arg	Ile	Asp	Asp	Ala	Ala	Ala				
			660					665					670						
Arg	Leu	Leu	Asp	Thr	Ser	Cys	Val	Arg	Asn	Asp	Arg	Leu	Leu	Glu	Ser				
			675				680					685							
Thr	Arg	Phe	Ile	Asp	Phe	Tyr	Arg	Tyr	Cys	Ser	Val	Ser	Arg	Lys	Asp				
	690					695					700								
Met	Tyr	Arg	Tyr	Ile	Ala	Glu	Arg	Val	Gly	Trp	Ser	Arg	Pro	Ala	Asp				
705					710					715					720				
Thr	Gly	Arg	Ser	Thr	Asn	Cys	Leu	Leu	Asn	Asp	Val	Gly	Ile	Tyr	Met				
				725					730					735					

His Lys Lys Gln Arg Gly Tyr His Asn Tyr Ser Leu Pro Tyr Ser Trp
 740 745 750
 Asp Val Arg Val Gly His Ile Pro Arg Glu Asp Ala Met Arg Glu Leu
 755 760 765
 Glu Asp Thr Asp Asp Ile Asp Glu Ala Lys Val Leu Gly Leu Leu Lys
 770 775 780
 Gln Ile Gly Tyr Asp Ser Ser Leu Ile Asp Thr Gln Ala Gly Asp Ala
 785 790 795 800
 Gln Leu Ile Ala Tyr Tyr Val Ala Ala Glu Glu Leu Asp Pro Val Ala
 805 810 815
 Leu Arg Asn Phe Ala Ala Ala Ile Leu Pro Glu Tyr Met Leu Pro Ser
 820 825 830
 Tyr Phe Val Arg Leu Asp Arg Met Pro Leu Thr Pro Asn Gly Lys Val
 835 840 845
 Asn Arg Arg Ala Leu Pro Arg Pro Glu Leu Lys Lys Asn Ala Ser Glu
 850 855 860
 Ala His Thr Glu Pro Ser Ser Ala Leu Glu Gln Glu Leu Val Gln Ile
 865 870 875 880
 Trp Lys Glu Val Leu Met Val Asp Lys Val Gly Val Arg Asp Asn Phe
 885 890 895
 Phe Glu Leu Gly Gly His Ser Leu Ser Ala Leu Met Leu Leu Tyr Ser
 900 905 910
 Ile Ala Glu Arg Tyr Gln Lys Met Val Ser Ile Gln Ala Phe Ser Val
 915 920 925
 Asn Pro Thr Ile Glu Gly Leu Ser Glu His Leu Val Ala
 930 935 940

<210> 30
 <211> 239
 <212> PRT
 <213> Xanthomonas albilineans

<400> 30

Met Asp Leu Gln Cys Ala Arg Ile Ala Ala Leu Cys Glu Gln Leu Lys
 1 5 10 15
 Leu Ala Arg Leu Ser Ser Asp Trp Gln Ala Leu Ala Gln Ala Ala Ala
 20 25 30
 Cys Glu Asp Ala Ser Tyr Phe Leu Glu Lys Val Leu Ala Ser Glu Gln
 35 40 45
 Leu Ala Arg Glu Glu Arg Lys Arg Thr Val Leu Thr Arg Leu Ala Arg
 50 55 60
 Met Pro Ser Ile Lys Thr Leu Glu Gln Phe Asp Trp Ala Gln Ala Gly


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<210> 31
<211> 286
<212> PRT
<213> Xanthomonas albilineans
<400> 31
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Met 1	Pro	Arg	Ile	Glu 5	Tyr	Cys	Ile	Ser	Met 10	Met	His	Arg	Arg	Lys 15	Pro
Thr	Thr	Asn 20	Arg	Ser	Val	Cys	Met	Arg 25	Asp	Ile	Glu	Arg	Thr 30	Ala	Leu
Trp	Val	Ala 35	Gly	Met	Arg	Ala	Leu 40	Glu	Ser	Glu	Arg	Glu 45	Gln	Ala	Leu
Phe 50	His	Asp	Pro	Phe	Ala	Arg 55	Arg	Leu	Ala	Gly	Asp 60	Glu	Phe	Val	Glu
Glu 65	Leu	Arg	Arg	Asn 70	Asn	Gln	Asn	Val	Pro	Met 75	Pro	Pro	Ala	Ile	Glu 80
Val	Arg	Thr	Arg	Trp 85	Leu	Asp	Asp	Lys 90	Ile	Met	Gln	Ala	Val	Ser 95	Glu
Gly	Ile	Gly	Gln 100	Val	Val	Ile	Leu 105	Ala	Ala	Gly	Met	Asp 110	Ala	Arg	Ala

Tyr Arg Leu Pro Trp Pro Ser Asp Thr Arg Val Tyr Glu Ile Asp His
 115 120 125
 Met Asp Val Leu Ser Asp Lys His Glu Lys Leu His Asp Ala Gln Pro
 130 135 140
 Val Cys Gln Arg Ile Ala Leu Pro Ile Asp Leu Arg Glu Asp Trp Pro
 145 150 155 160
 Gln Ala Leu Lys Glu Ser Gly Phe Val Gly Ser Ala Ala Thr Leu Trp
 165 170 175
 Leu Val Glu Gly Leu Leu Cys Tyr Leu Ser Ala Glu Ala Val Met Leu
 180 185 190
 Leu Phe Ala Arg Ile Asp Ala Leu Ser Ala Lys Gly Ser Ser Val Leu
 195 200 205
 Phe Asp Val Ile Gly Leu Ser Met Leu Asn Ser Pro Asn Ala Arg Val
 210 215 220
 Leu His Ala Met Ala Arg Gln Phe Gly Thr Asp Glu Pro Glu Ser Leu
 225 230 235 240
 Ile Gln Pro Leu Gly Trp Glu Pro Gly Val Leu Thr Ile Ala Ala Ala
 245 250 255
 Gly Gln Gln Met Gly Arg Trp Pro Phe Pro Val Ala Pro Arg Gly Thr
 260 265 270
 His Gly Val Pro Gln Ser Tyr Leu Val His Ala Leu Lys Arg
 275 280 285

<210> 32
 <211> 765
 <212> PRT
 <213> Xanthomonas albilineans

<400> 32

Met Arg Arg Ser Pro Tyr Pro Arg Thr Leu Met Asp Ser Pro Leu Thr
 1 5 10 15
 Asn Leu Pro Met His Ser Gly Thr Glu Leu Asp Leu Arg Trp Ser Val
 20 25 30
 Gly Gln Thr Arg Pro Gly Arg Asn Glu Ala Tyr Ala Arg Gln Trp Thr
 35 40 45
 Thr Leu Leu His Gln Trp Arg Arg Asp Tyr Pro Gly Leu Arg Ile Asp
 50 55 60
 Val Ser Asp Thr Pro Ile Gly Gln His Ile Thr Ile Asp Tyr Ala Ala
 65 70 75 80
 Pro Tyr Pro Cys Gly Ser Phe Gly Ser Leu Leu Arg Glu Tyr Ala Arg
 85 90 95
 Leu Gly Lys Leu Ala Gly Leu Ile Cys Asp Tyr Leu Lys His Arg His
 100 105 110

Gln Ile Val Leu Ser Glu Ser Pro Pro Gly Ala Asn Thr Leu Ala Leu
 115 120 125
 Asp Leu Gly Arg Ile Glu Glu Pro Lys Gln Leu Asp Arg Leu Gln Gly
 130 135 140
 Ala Leu Gly Met Ala Leu Glu Ala Leu Ala Thr Arg Arg Ser Asp Gly
 145 150 155 160
 Leu Leu Leu Trp His Ala Asp His Arg Gln Arg Asn Leu Pro Asp Leu
 165 170 175
 Arg Asp Ser Ala Val Cys Gly Ser Ala Ala Gln Ile Ser Leu Pro Ala
 180 185 190
 Leu Ser Cys Val Glu Asp Leu Ile Glu Val Asp Thr Ser Leu Leu Ala
 195 200 205
 Cys Asp His Gly Lys Leu Cys Gln Ile Ala Ser His Leu Pro Ala Ser
 210 215 220
 Trp Phe Ala Arg Ser Thr Asp Gly Pro Met Pro Ser Trp Ser Asp Ala
 225 230 235 240
 Ser Thr Ala Val Phe Ala Cys Ala Pro Ile Gly Phe Leu Pro Ser Val
 245 250 255
 Gln Val Asn Val Cys Ala Gln Ile Phe Ser Ala Ala His Leu Ala Ser
 260 265 270
 Thr Ala Gln Met Ile Asp Pro Leu Arg Gln Gln Ala Phe Ser Tyr Arg
 275 280 285
 Gln Leu Arg Ser Arg Ala Ala Thr Tyr Ala Arg His Leu Ser Leu Leu
 290 295 300
 Gly Leu Gln Ser Gly Asp Ala Val Ala Leu Ile Ala Ile Asp Ser Leu
 305 310 315 320
 Ala Gly Val Ala Leu Met Leu Ala Cys Leu Ala Gly Gly Leu Val Phe
 325 330 335
 Ala Pro Ile Asn Glu Leu Val Ser Leu Val His Phe Glu Thr Thr Leu
 340 345 350
 Lys Thr Ile Lys Pro Arg Leu Val Leu Ile Asp Ala Glu Leu Pro Pro
 355 360 365
 Ser His His Ala Ala Leu Arg His Leu Pro Thr Leu Glu Leu Thr Ser
 370 375 380
 Leu Met Pro Val Ile Glu Asn Asp Glu Leu Val Val Ala Pro Cys Ser
 385 390 395 400
 Ala Asp Ala Pro Ala Val Met Ile Cys Thr Ser Gly Ser Thr Gly Thr
 405 410 415
 Pro Lys Ala Val Thr His Ser His Ala Asp Phe Met His Cys His Leu
 420 425 430

Asn Tyr Gln Gln Ala Val Leu Gly Leu Arg Ser Asp Asp Val Met Tyr
 435 440 445
 Thr Pro Ser Arg Leu Phe Phe Ala Tyr Gly Leu Asn Asn Leu Met Leu
 450 455 460
 Ser Leu Leu Ala Gly Val Ser His Val Ile Ala Ala Pro Leu Ser Val
 465 470 475 480
 Arg Gln Ile Ala Gln Thr Ile His Thr Tyr His Val Thr Val Leu Leu
 485 490 495
 Ala Val Pro Ala Val Phe Lys Leu Leu Leu Ala Glu Ala Ala Pro Asp
 500 505 510
 Ala Val Trp Pro Ala Leu Arg Leu Cys Ile Ser Ala Gly Glu Ser Leu
 515 520 525
 Pro Ala Arg Leu Gly His Ala Ile Ser Thr Arg Trp Gln Val Glu Val
 530 535 540
 Leu Asp Gly Ile Gly Cys Thr Glu Val Leu Ser Thr Phe Ile Ser Asn
 545 550 555 560
 Arg Pro Gly His Ala Leu Met Gly Cys Thr Gly Thr Pro Val Pro Gly
 565 570 575
 Phe Val Val Lys Leu Val Asn Lys Gln Gly Glu Ile Cys Arg Ile Gly
 580 585 590
 Glu Val Gly Ser Leu Trp Val Arg Gly Asn Thr Leu Thr Arg Gly Tyr
 595 600 605
 Val Gly Asp Pro Ile Leu Ser Ala Gln Leu Phe Val Asp Gly Trp Phe
 610 615 620
 Asp Thr Arg Asp Leu Phe Phe Ala Asp Ala Lys Gly Arg Phe His Asn
 625 630 635 640
 Leu Gly Arg Met Gly Ser Ala Ile Lys Ile Asn Gly Cys Trp Leu Ser
 645 650 655
 Pro Glu Thr Leu Glu Ser Val Ile Gln Thr His Ala Cys Val Lys Glu
 660 665 670
 Cys Ala Ile Cys Leu Ile Glu Asp Glu Phe Gly Leu Pro Arg Pro Ala
 675 680 685
 Ala Phe Val Val Pro Val Asp Ala Ser Ile Asp Thr Gly Ala Leu Trp
 690 695 700
 Ala Ala Leu Arg Ala Leu Cys Lys Asn Ala Leu Gly Lys His His Tyr
 705 710 715 720
 Pro His Leu Phe Val Glu Val Ser Thr Ile Pro Arg Thr Cys Ser Gly
 725 730 735
 Lys Val Ile Arg Pro Ala Leu Leu Glu Thr Leu Ala Ser Ala Lys His
 740 745 750
 Leu Gln Ser His Leu Phe Phe Val Gly His Ala Arg Thr

755

760

765

<210> 33
 <211> 330
 <212> PRT
 <213> Xanthomonas albilineans

<400> 33

Met His Thr Asn Ala Asp Leu Pro Leu Thr Ile Lys Ala Asp Ser Ala
 1 5 10 15
 Glu Ala Thr Leu Thr Asp Trp Asn Ala Thr His Arg Ala Thr Trp Pro
 20 25 30
 Thr Leu Leu Trp Gln His Arg Ala Leu Leu Phe Arg Gly Phe Ala His
 35 40 45
 Pro Gly Gly Leu Glu Gln Ile Ser Arg Cys Phe Phe Asp Glu Arg Leu
 50 55 60
 Ala Tyr Thr Tyr Arg Ser Thr Pro Arg Thr Asp Val Gly Gln His Val
 65 70 75 80
 Tyr Thr Ala Thr Glu Tyr Pro Arg Gln Leu Ser Ile Ala Gln His Cys
 85 90 95
 Glu Asn Ala Tyr Gln Arg Val Trp Pro Met Lys Leu Leu Phe His Cys
 100 105 110
 Val Gln Pro Ala Ser Glu Gly Gly Cys Thr Pro Leu Ala Asp Met Leu
 115 120 125
 Lys Val Thr Ala Ala Ile Asp Pro Gln Val Arg Glu Ile Phe Ala Arg
 130 135 140
 Lys Gln Val Arg Tyr Val Arg Asn Tyr Arg Ala Gly Val Asp Leu Pro
 145 150 155 160
 Trp Glu Asp Val Phe Asn Thr Arg Asn Lys Gln Glu Val Glu Ala Tyr
 165 170 175
 Cys Ala Arg Asn Asp Met Gln Cys Glu Trp Thr Gly Asp Gly Leu Arg
 180 185 190
 Thr Ser Gln Ile Cys Arg Ala Phe Ala Cys His Pro Ala Thr Gly Asp
 195 200 205
 Glu Val Trp Phe Asn Gln Ala His Leu Phe His Tyr Thr Ala Leu Glu
 210 215 220
 Ala Ala Ala Gln Lys Met Met Leu Ser Phe Phe Gly Glu Gln Gly Leu
 225 230 235 240
 Pro Arg Asn Ala Tyr Phe Gly Asp Gly Thr Pro Ile Asp Pro Ala Met
 245 250 255
 Leu Asp His Val Arg Thr Val Phe Ala Gln His Lys Ile His Phe Asp
 260 265 270
 Trp His Arg Asp Asp Val Leu Leu Ile Asp Asn Met Leu Val Ser His

275	280	285
Gly Arg Glu Pro Tyr Glu Gly Ser Arg Lys Ile Leu Val Cys Met Ala		
290	295	300
Glu Pro Tyr Ser Pro Glu Gln Ser Ser Pro Asp Ile Ala Ala Arg Ser		
305	310	315 320
Asp Gly Glu Ala Met Leu Lys Leu His Val		
325	330	

<210> 34
 <211> 1959
 <212> PRT
 <213> Xanthomonas albilineans

<400> 34

Met Lys Leu Ser Ser Met Ser Leu Leu Asp Ala Glu Asp Val Ala Leu	
1 5 10 15	
Thr Ala Ala Ser Pro Asp Thr Ala Leu Ala Leu Asp Trp Ser Arg Ser	
20 25 30	
Val Leu Asp Leu Phe Asp Ala Gln Val Ala Leu His Ala Glu Glu Leu	
35 40 45	
Ala Cys Ala Asp Gln His Arg Gln Leu Ser Tyr Ala Gln Leu Asp Gln	
50 55 60	
His Ala Asn Arg Leu Ala His Cys Leu Ile Glu Arg Gly Leu Arg Pro	
65 70 75 80	
Gln Glu Arg Val Ala Leu Trp Phe Gly Arg Ser Pro Asp Phe Leu Ile	
85 90 95	
Ala Leu Leu Gly Val Leu Lys Ala Gly Gly Cys Tyr Val Pro Leu Asp	
100 105 110	
Pro His Tyr Pro Thr Thr Tyr Ile Gln Gln Ile Leu Asp Asp Ala Gln	
115 120 125	
Pro Arg Leu Leu Leu Cys Gly Lys Asp Ile Asp Gly Gln Leu Ile Gln	
130 135 140	
Val Pro Arg Leu Arg Leu Asp Asp Ala Ala Ile Ala Arg Gln Pro His	
145 150 155 160	
Thr Pro Leu Pro His Ala Leu His Pro Ala Gln Leu Ala Tyr Val Met	
165 170 175	
Tyr Thr Ser Gly Ser Thr Gly Arg Pro Lys Gly Val Met Val Pro His	
180 185 190	
Arg Gln Ile Leu Asn Trp Leu His Ala Leu Trp Ala Arg Ala Pro Phe	
195 200 205	
Glu Ala Gly Glu Arg Val Ala Gln Lys Thr Ser Ile Ala Phe Ala Ile	
210 215 220	

Ser Val Lys Glu Leu Leu Ala Gly Leu Leu Ala Gly Val Pro Gln Val
 225 230 235 240
 Phe Ile Asp Glu Asp Thr Val Arg Asp Ile Pro Ala Phe Val Arg Ala
 245 250 255
 Leu Glu Thr Trp Gln Ile Thr Arg Leu Tyr Thr Phe Pro Ser Gln Leu
 260 265 270
 Asn Ala Leu Leu Asp His Val Ala Glu Thr Pro Gln Arg Leu Ala Arg
 275 280 285
 Leu Arg Gln Leu Phe Val Ser Ile Glu Pro Cys Pro Ala Glu Leu Leu
 290 295 300
 Gln Arg Leu Arg Thr Leu Leu Pro Ala Cys Thr Ala Trp Tyr Ile Tyr
 305 310 315 320
 Gly Cys Thr Glu Ile Asn Asp Met Thr Tyr Cys Asp Pro Ala Glu Gln
 325 330 335
 His Ser Gly Ser Gly Phe Val Pro Val Gly Arg Pro Ile Ala Asn Thr
 340 345 350
 Lys Val His Val Leu Asp Glu Gln Leu Arg Pro Leu Pro Pro Gly Ile
 355 360 365
 Met Gly Glu Val His Ile Glu Ser Leu Gly Ile Thr His Gly Tyr Trp
 370 375 380
 Arg Gln Gly Gly Leu Thr Ala Ala Arg Phe Ile Ala Asn Pro Tyr Gly
 385 390 395 400
 Pro Pro Gly Ser Arg Leu Tyr Arg Thr Gly Asp Met Ala Arg Leu Leu
 405 410 415
 Asp Asn Gly Thr Leu Glu Leu Leu Gly Arg Arg Asp Tyr Glu Val Lys
 420 425 430
 Val Arg Gly Tyr Arg Val Asp Val Arg Gln Val Glu Lys Ala Leu Ala
 435 440 445
 Ala His Leu Gln Val Ala Glu Ala Ala Val Ile Gly Trp Pro Gln Gly
 450 455 460
 Ser Pro Thr Pro Glu Leu Leu Ala Tyr Val Val Pro Arg Gln Gly Val
 465 470 475 480
 Leu Asn Leu Asp Glu Leu Arg Lys Leu Leu Gln Glu Arg Leu Pro Thr
 485 490 495
 Tyr Met Leu Pro Thr Arg Phe Gln Ser Leu Pro Ala Leu Pro Arg Leu
 500 505 510
 Pro Asn Gly Lys Leu Asp Thr Leu Ser Leu Pro Glu Pro Gln Ala Ala
 515 520 525
 Ser Ser Asp Ser Asp Tyr Leu Ala Pro Arg Ser Glu Val Glu Ile Thr
 530 535 540
 Leu Ala Lys Leu Trp Ser Glu Leu Leu Thr Pro Ala Gln Ala Ala Pro

545		550		555		560
Leu Arg Val Ser	Leu Asn Asp Asn Phe Phe Asn Leu Gly Gly His Ser	565		570		575
Leu Leu Ala Thr	Gln Leu Phe Ser Arg Ile Arg Gln Ser Phe Asp Ile	580		585		590
Glu Val Arg Val	Asn Thr Leu Phe Glu Ser Pro Val Leu Glu Asp Phe	595		600		605
Ala Arg Val Val	Asn Glu Ala Arg Gln Gln Gln Ala Pro Thr Gly Gly	610		615		620
Asn Thr Ile Ser	Ser Arg Ala Val Arg Asp Ala Pro Val Pro Leu Ser	625		630		635
Tyr Gln Gln Glu	Arg Leu Trp Phe Val His Glu His Met Pro Glu Gln	645		650		655
Arg Thr Ser Tyr	Asn Val Ala Phe Ala Cys His Leu Arg Ser Ala Asp	660		665		670
Phe Ser Met Ser	Ala Leu Arg Glu Ala Ile Gln Ala Leu Val Ala Arg	675		680		685
His Glu Thr Leu	Arg Thr Arg Ile Ala Thr Cys Ala Gly Gly Asp Tyr	690		695		700
Pro Ser Gln His	Ile Ala Asp Ala Met Gln Val Pro Val Pro Cys Ile	705		710		715
Thr Ala Thr Pro	Ala Glu Val Pro Arg Leu Val Ala Glu His Ala Ala	725		730		735
His Val Phe Asp	Leu Ala His Gly Pro Leu Leu Lys Val Ser Val Leu	740		745		750
Arg Val Ser Asp	Asp Tyr His Val Phe Leu Met Asn Met His His Ile	755		760		765
Ile Cys ^h Asp Gly	Trp Ser Ile Asn Leu Ile Phe His Asp Leu Arg Ala	770		775		780
Phe Tyr Ile Ala	Ala Leu Gln Gln Thr Pro Pro Ala Leu Pro Pro Leu	785		790		795
Leu Leu Gln Tyr	Ala Asp Tyr Ala Thr Trp Gln Arg Val Gln Asp Phe	805		810		815
Ser Ala Asp Leu	Asp Tyr Trp Lys Gln Arg Leu His Gly Tyr Glu Glu	820		825		830
Gly Leu Ala Leu	Pro Tyr Asp Phe Pro Arg Pro Ala Asn Arg Ala Trp	835		840		845
Arg Ala Gly Ile	Leu His Leu Thr Tyr Pro Asp Ala Leu Ala Ala Arg	850		855		860
Leu Ala Ala Phe	Ser Gln Glu Arg Arg Val Thr Leu Phe Met Thr Leu	865		870		875
						880

Met Ala Ser Leu Ala Ile Val Leu His Gln Tyr Thr Gly Arg Arg Glu
 885 890 895
 Leu Cys Leu Gly Thr Thr Ser Ala Gly Arg Asp Gln Leu Glu Thr Glu
 900 905 910
 Asn Leu Ile Gly Phe Phe Val Asn Ile Leu Ala Val Arg Leu Asn Leu
 915 920 925
 Gly Ser His Ala Phe Ala Glu Asp Phe Leu Gln His Val Arg Gln Gln
 930 935 940
 Val Leu Asp Ala Tyr Ala His Arg Ala Leu Pro Phe Glu His Val Leu
 945 950 955 960
 Ser Ala Leu Lys Lys Pro Arg Asp Ser Ser Gln Ile Pro Leu Val Pro
 965 970 975
 Ile Met Leu Arg His Gln Asn Phe Ala Thr Glu Gly Val Asn Ala Phe
 980 985 990
 Ala Gln Ile Phe Leu Ser Ala Gln Met Glu Phe Gly Glu Arg Thr Thr
 995 1000 1005
 Pro Asn Glu Leu Asp Leu Gln Phe Ile Gly Asp Gly Ser His Leu
 1010 1015 1020
 Glu Val Thr Val Glu Tyr Ala Ala Glu Leu Phe Ser Ala Ala Thr
 1025 1030 1035
 Val Gln Arg Met Leu Ala His His Gln Arg Val Leu Glu Arg Met
 1040 1045 1050
 Leu Glu Glu Pro Arg Cys Arg Leu Ser Asp Phe Ser Leu Pro Val
 1055 1060 1065
 Ala Arg Thr Glu Phe Thr Pro His Thr Leu Asp Thr Ser Arg Ser
 1070 1075 1080
 Val Leu Asp Leu Phe Asp Ala Gln Val Ala Leu His Ala Glu Glu
 1085 1090 1095
 Leu Ala Cys Ala Asp Gln His Arg Gln Leu Ser Tyr Ala Gln Leu
 1100 1105 1110
 Asp Gln His Ala Asn Arg Leu Ala His Cys Leu Ile Glu Arg Gly
 1115 1120 1125
 Leu Arg Pro Gln Glu Arg Val Ala Leu Trp Phe Gly Arg Ser Pro
 1130 1135 1140
 Asp Phe Leu Ile Ala Leu Leu Gly Val Leu Lys Ala Gly Gly Cys
 1145 1150 1155
 Tyr Val Pro Leu Asp Pro His Tyr Pro Thr Thr Tyr Ile Gln Gln
 1160 1165 1170
 Ile Leu Asp Asp Ala Gln Pro Arg Leu Leu Leu Cys Gly Lys Asp
 1175 1180 1185

Ile	Asp	Gly	Gln	Leu	Ile	Gln	Val	Pro	Arg	Leu	Arg	Leu	Asp	Asp
1190						1195					1200			
Ala	Ala	Ile	Ala	Arg	Gln	Pro	His	Thr	Pro	Leu	Pro	His	Ala	Leu
1205						1210					1215			
His	Pro	Ala	Gln	Leu	Ala	Tyr	Val	Met	Tyr	Thr	Ser	Gly	Ser	Thr
1220						1225					1230			
Gly	Arg	Pro	Lys	Gly	Val	Met	Val	Pro	His	Arg	Gln	Ile	Leu	Asn
1235						1240					1245			
Trp	Leu	His	Ala	Leu	Trp	Ala	Arg	Ala	Pro	Phe	Glu	Ala	Gly	Lys
1250						1255					1260			
Arg	Val	Ala	Gln	Lys	Thr	Ser	Ile	Ala	Phe	Ala	Ile	Ser	Val	Lys
1265						1270					1275			
Glu	Leu	Leu	Ala	Gly	Leu	Leu	Ala	Gly	Val	Pro	Gln	Val	Phe	Ile
1280						1285					1290			
Asp	Glu	Asp	Thr	Val	Arg	Asp	Ile	Pro	Ala	Phe	Val	Arg	Ala	Leu
1295						1300					1305			
Glu	Thr	Trp	Gln	Ile	Thr	Arg	Leu	Tyr	Thr	Phe	Pro	Ser	Gln	Leu
1310						1315					1320			
Asn	Ala	Leu	Leu	Asp	His	Val	Ala	Glu	Thr	Pro	Gln	Arg	Leu	Ala
1325						1330					1335			
Arg	Leu	Arg	Gln	Leu	Phe	Val	Ser	Ile	Glu	Pro	Cys	Pro	Ala	Glu
1340						1345					1350			
Leu	Leu	Gln	Arg	Leu	Arg	Thr	Leu	Leu	Pro	Ala	Cys	Thr	Ala	Trp
1355						1360					1365			
Tyr	Ile	Tyr	Gly	Cys	Thr	Glu	Ile	Asn	Asp	Met	Thr	Tyr	Cys	Asp
1370						1375					1380			
Pro	Ala	Glu	Gln	His	Ser	Gly	Ser	Gly	Phe	Val	Pro	Val	Gly	Arg
1385						1390					1395			
Pro	Ile	Ala	Asn	Thr	Lys	Val	His	Val	Leu	Asp	Glu	Gln	Leu	Arg
1400						1405					1410			
Pro	Leu	Pro	Pro	Gly	Ile	Met	Gly	Glu	Val	His	Ile	Glu	Ser	Leu
1415						1420					1425			
Gly	Ile	Thr	His	Gly	Tyr	Trp	Arg	Gln	Gly	Gly	Leu	Thr	Ala	Ala
1430						1435					1440			
Arg	Phe	Ile	Ala	Asn	Pro	Tyr	Gly	Pro	Pro	Gly	Ser	Arg	Leu	Tyr
1445						1450					1455			
Arg	Thr	Gly	Asp	Met	Ala	Arg	Leu	Leu	Asp	Asn	Gly	Thr	Leu	Glu
1460						1465					1470			
Leu	Leu	Gly	Arg	Arg	Asp	Tyr	Glu	Val	Lys	Val	Arg	Gly	Tyr	Arg
1475						1480					1485			
Val	Asp	Val	Arg	Gln	Val	Glu	Lys	Ala	Leu	Ala	Ala	His	Leu	Gln
1490						1495					1500			

Val	Ala	Glu	Ala	Ala	Val	Ile	Gly	Trp	Pro	Gln	Gly	Ser	Pro	Thr
1505						1510					1515			
Pro	Glu	Leu	Leu	Ala	Tyr	Val	Val	Pro	Arg	Gln	Gly	Val	Leu	Asn
1520						1525					1530			
Leu	Asp	Glu	Leu	Arg	Lys	Leu	Leu	Gln	Glu	Arg	Leu	Pro	Thr	Tyr
1535						1540					1545			
Met	Leu	Pro	Thr	Arg	Phe	Gln	Ser	Leu	Pro	Ala	Leu	Pro	Arg	Leu
1550						1555					1560			
Pro	Asn	Gly	Lys	Leu	Asp	Thr	Leu	Ser	Leu	Pro	Glu	Pro	Gln	Ala
1565						1570					1575			
Ala	Ser	Ser	Asp	Ser	Asp	Tyr	Leu	Ala	Pro	Arg	Ser	Glu	Val	Glu
1580						1585					1590			
Ile	Thr	Leu	Ala	Lys	Leu	Trp	Ser	Glu	Leu	Leu	Thr	Pro	Ala	Gln
1595						1600					1605			
Ala	Ala	Pro	Leu	Arg	Val	Ser	Leu	Asn	Asp	Asn	Phe	Phe	Asn	Leu
1610						1615					1620			
Gly	Gly	His	Ser	Leu	Leu	Ala	Thr	Gln	Leu	Phe	Ser	Arg	Ile	Arg
1625						1630					1635			
Gln	Ser	Phe	Asp	Ile	Glu	Val	Arg	Val	Asn	Thr	Leu	Phe	Glu	Ser
1640						1645					1650			
Pro	Val	Leu	Glu	Asp	Phe	Ala	Ala	Val	Val	Glu	Arg	Gly	Met	Arg
1655						1660					1665			
Gln	Ser	Gln	Ala	Gly	Ser	Met	Pro	Val	Ser	Leu	Ile	Val	Pro	Leu
1670						1675					1680			
Ser	Leu	Arg	Thr	Glu	Arg	Ala	Ala	Val	Tyr	Ala	Ile	His	Pro	Ile
1685						1690					1695			
Gly	Gly	Gln	Ile	His	Cys	Tyr	Ile	Asp	Leu	Ala	Ala	Ala	Leu	Gly
1700						1705					1710			
His	Ser	Ala	Arg	Val	Tyr	Gly	Leu	Gln	Cys	Glu	Pro	Val	Arg	Arg
1715						1720					1725			
Phe	Ala	His	Leu	Ser	Asp	Leu	Ala	Ala	His	Tyr	Cys	Asp	Ala	Leu
1730						1735					1740			
Leu	Ala	Gly	Pro	Thr	Gly	Ala	Pro	Tyr	Arg	Leu	Leu	Gly	Trp	Ser
1745						1750					1755			
Ser	Gly	Gly	Val	Leu	Ala	Leu	Ala	Val	Ala	Glu	Gln	Leu	Gln	Arg
1760						1765					1770			
Arg	Gly	Leu	Arg	Val	Asp	Tyr	Val	Gly	Leu	Leu	Asp	Ser	Ser	Leu
1775						1780					1785			
Ile	Pro	Val	His	Ala	Arg	Glu	Pro	Arg	Gln	Leu	Thr	Phe	Val	Ala
1790						1795					1800			

Ala Leu Asn Thr Leu Ala Ala Leu Ala Lys Arg Gly Phe Glu Gln
 1805 1810 1815

Ala Glu Ile Asp Glu Ala Arg Gln Leu Leu Phe Ala Asp Gly Asp
 1820 1825 1830

Asp Glu His Val Phe Asp Tyr Ser Arg His Gln Ala Ser Leu Asp
 1835 1840 1845

Lys Leu Leu Ala His Leu Arg Phe Thr Leu Glu Ser Arg Met Trp
 1850 1855 1860

Pro Pro Leu Ala Glu Gln Leu Arg Val Thr Arg Tyr His Leu Gly
 1865 1870 1875

Leu Leu Ala Gly Phe Glu Pro Gln Cys Leu Gln Pro Asn Ala His
 1880 1885 1890

Leu Tyr Gln Ala Gln Thr Ala Val His Val Ser Tyr Ala Asp Met
 1895 1900 1905

Ser Lys Pro Arg Gly Gly Ser Glu Val Leu Pro Asp Ile Thr Gly
 1910 1915 1920

Tyr Val Pro Leu Ser Gln Ile Lys Ser Ala Ala Gly Asn His Tyr
 1925 1930 1935

Ser Met Leu Gln Gly Asp Pro Leu Arg Glu Leu Ala Arg Met Leu
 1940 1945 1950

Val Thr Asp Leu Asp Ala
 1955

<210> 35
 <211> 83
 <212> PRT
 <213> Xanthomonas albilineans

<400> 35

Met Thr Phe Glu Glu Gln Ala Tyr Leu Val Leu Ile Asn Asp Glu Leu
 1 5 10 15

Gln Tyr Ser Leu Trp Pro Ser Asp Leu Glu Val Pro Pro Gly Trp Arg
 20 25 30

Lys Glu Gly Tyr Ala Gly Gly Lys Asp Glu Cys Met Ala Tyr Ile Asp
 35 40 45

Glu Thr Trp Thr Asp Met Arg Pro Leu Ser Leu Arg Glu Leu Asp Asp
 50 55 60

Lys Asn Leu Gly Asp Ala Ser Ser Pro Asp Gly Ser Gly Phe Glu Ser
 65 70 75 80

Ser Tyr Ser

<210> 36

<211> 315
 <212> PRT
 <213> Xanthomonas albilineans

<400> 36

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Met Gly Cys Ala Cys Leu Pro His Tyr Leu Glu Lys Gln Asp Leu Ser
1          5          10          15

Ala Leu Asp Asp Ala Leu Ala Gly Val Arg Leu Ser Gln Tyr Cys Thr
          20          25          30

Thr Asp Gly Arg Gln Leu Glu Leu Tyr Trp Leu Gly Ala Gln Ala Ser
          35          40          45

Pro Lys Leu Val Leu Leu Pro Pro Tyr Gly Met Ser Tyr Leu Leu Leu
          50          55          60

Ser Arg Leu Ala Gln Arg Leu Ala Arg His Phe His Val Leu Cys Trp
65          70          75          80

Glu Ser Ile Gly Cys Pro Asn Ala Gln Thr Ser Val Thr Ala Glu Asp
          85          90          95

Phe Asp Leu Asp Arg Gln Ala Ala Thr Leu Leu Gly Ile Leu His Gln
          100          105          110

His Asp Tyr Ala Asp Cys His Phe Val Gly Trp Cys Gln Ala Ala Gln
          115          120          125

Leu Ala Val His Ala Ile Ala Leu His Gly Phe Ala Pro Arg Ser Met
          130          135          140

Ala Trp Val Ala Pro Ala Gly Leu Leu Pro Pro Ile Val Lys Ser Glu
145          150          155          160

Phe Glu Arg Cys Ala Leu Pro Ile Tyr Leu Gln Ile Glu Arg His Gly
          165          170          175

Leu Glu Gln Ala Lys Lys Leu Ala Ala Ile Leu Asp Lys Tyr Arg Gly
          180          185          190

Gln Pro Leu Arg Gly Asp Asp Leu Ala Glu Lys Leu Thr Met Leu His
          195          200          205

Leu Ala Asp Pro Ala Ser Thr Leu Val Phe Ser Arg Tyr Met Arg Ala
          210          215          220

Tyr Glu Glu Asn Lys Gln Ser Val Gln Ala Leu Leu Pro Thr Ala Leu
225          230          235          240

Gly Arg His Pro Thr Leu Ile Val His Cys Lys Asp Asp Ser Phe Ser
          245          250          255

His Tyr Ser Ala Ser Val Gln Leu Ala Arg His Asp Pro Ser Leu Arg
          260          265          270

Leu Asp Leu Leu Asp His Gly Gly His Leu Gln Leu Phe Asn Asp Pro
          275          280          285

Gly Ala Val Ala Gln Arg Ile Ile Asp Phe Ile Gly Leu Thr Val Gly

```

290

295

300

Glu Val Ala Pro Thr Ser Met His Ser Ala Ala
 305 310 315

<210> 37

<211> 451

<212> PRT

<213> Xanthomonas albilineans

<400> 37

Met Tyr Ile Pro Asn Asn Ile Asp Leu Asp Pro His Ser Ala Leu Val
 1 5 10 15

Arg Gln Leu Thr Ser Tyr Gln Val Arg Phe Leu Gln Trp Trp Arg Leu
 20 25 30

Arg Gly Pro Ser Glu Phe His Asp Arg Glu Met Asn Leu Arg Met Pro
 35 40 45

Thr Gly Gly Val Lys Gly Ser Glu Trp Thr Arg Tyr His Arg Met Arg
 50 55 60

Pro Ser Asp Tyr Arg Trp Gly Val Phe Met Met Pro Pro Asp Arg Asn
 65 70 75 80

Thr Val Val Phe Gly Glu Arg Lys Gly Gln Val Ala Trp Ser Cys Val
 85 90 95

Pro Glu Glu Tyr Arg Asp Leu Leu Leu Asp His Val Thr Val Gln Gly
 100 105 110

Asp Val Glu Asn Ala Ala Val Glu Gln Ser His Glu Leu Thr Gln Met
 115 120 125

Val Pro Ser Ala Ile Asp Leu Glu His Leu Phe Gln Phe Phe Leu Glu
 130 135 140

Glu Gly Arg His Thr Trp Ala Met Ser His Leu Leu Ile Glu Tyr Phe
 145 150 155 160

Gly Ser Asp Gly Ala Asp Ala Ala Glu Gly Leu Leu Gln Arg Met Ser
 165 170 175

Gly Asp Ala Gln Asn Pro Arg Leu Leu Asp Ala Phe Asn Tyr His Thr
 180 185 190

Glu Asp Trp Leu Ser His Phe Met Trp Cys Phe Phe Ala Asp Arg Val
 195 200 205

Gly Lys Tyr Gln Ile Gln Ala Val Thr Gln Ser Ala Phe Leu Pro Leu
 210 215 220

Ala Arg Thr Ala Arg Phe Met Met Phe Glu Glu Pro Leu His Ile Lys
 225 230 235 240

Phe Gly Val Asp Gly Leu Glu Arg Val Leu Tyr Arg Ser Ala Glu Ile
 245 250 255

Thr Leu Arg Glu Asp Thr His Ala Ile Phe Asp Ala Gly Ala Ile Pro
 260 265 270
 Leu Pro Val Val Gln Lys Tyr Leu Asn Tyr Trp Leu Pro Lys Ile Phe
 275 280 285
 Asp Leu Phe Gly His Asp Val Ser Glu Arg Ser Arg Val Leu Tyr Gln
 290 295 300
 Ala Gly Ile Arg Ser Pro Arg Asn Phe Asp Lys Leu Glu Gly Thr Glu
 305 310 315 320
 Val Ala Val Asp Val Arg Cys Glu Asp Arg Leu Val Ser Ser Thr Ala
 325 330 335
 Pro Ala Glu Leu Ala Ile Asn Ala Val Met Arg Arg Gln Tyr Ile Ala
 340 345 350
 Glu Val Gly Ala Ile Ile Gly Arg Trp Asn Gln Gln Leu Arg Arg Leu
 355 360 365
 Gly Leu Ala Phe Glu Leu Gln Leu Pro His Glu Arg Phe His Arg Asp
 370 375 380
 Phe Gly Pro Cys Lys Gly Leu Ala Phe Asp Leu Asp Gly Asn Pro Val
 385 390 395 400
 His Asp Ala Asp Gly Gln Arg Leu Ala Ala Leu Leu Pro Thr Pro Gln
 405 410 415
 Asp Leu Ala Gly Val Arg Gly Leu Met Gly Arg Glu Leu Gly Glu Gly
 420 425 430
 Arg Thr Ala Val Trp Leu Ala Pro Ala Gly Ala Ser Leu Asp Lys Leu
 435 440 445
 Met Pro Ala
 450

<210> 38
 <211> 317
 <212> PRT
 <213> Xanthomonas albilineans

<400> 38

Met Asn Ser Tyr Val Gly Cys Gln Lys Leu Glu Thr Asp Gly Asp Ala
 1 5 10 15
 Ser Arg Val Val Pro Met Trp Val Met Tyr Pro Thr Ala Thr Pro Ser
 20 25 30
 Arg Asp Thr Ala Met Gly Pro Tyr Thr Leu Asp Val Ala Leu Gly Ala
 35 40 45
 Pro Ile Glu Ala Gly Pro Phe Pro Leu Ala Val Ile Ser His Gly Thr
 50 55 60
 Arg Ser Ala Gly Leu Val Phe Arg Thr Leu Ala His Tyr Leu Ala Arg
 65 70 75 80

His Gly Phe Ile Val Ala Leu Pro Glu His Pro Gly Asp Asn Leu Phe
 85 90 95
 Gln His Gln Leu Glu Tyr Ser Tyr Gln Asn Leu Glu Asp Arg Pro Arg
 100 105 110
 His Ile Arg Ala Val Ile Asp Thr Leu Thr Gly His Ala Gln Phe Gly
 115 120 125
 Pro Ala Ile Gln Ala His Asn Val Ala Val Ile Gly His Ser Val Gly
 130 135 140
 Gly Tyr Thr Ala Leu Ala Ile Ala Gly Gly Glu Pro His Thr Gly Phe
 145 150 155 160
 Met Val Asp Phe Ala His Arg Pro Glu His Ala Glu Gln Pro Ala Trp
 165 170 175
 Thr Ala Leu Val Arg Gln Asn Arg Val Pro Ile Arg Ala Val Pro Val
 180 185 190
 Thr Ala Asp Pro Arg Val Arg Ala Val Val Ala Leu Ala Pro Asp Phe
 195 200 205
 Ser Leu Tyr Met His Glu Asp Ala Leu Ala Lys Val Glu Val Pro Val
 210 215 220
 Leu Leu Ile Val Gly Glu Lys Asp Gln Trp Ala His Glu Thr Ile Val
 225 230 235 240
 Ala Thr Arg Thr Ala Leu Gly Asn Asp Gly Arg Leu Glu Ala Arg Val
 245 250 255
 Val Pro Asn Ala Gly His Tyr Ala Phe Ile Ser Val Phe Pro Glu Ala
 260 265 270
 Met Lys Ala Arg Val Gly Glu Ala Ala Ile Asp Pro Pro Gly Phe Asp
 275 280 285
 Arg Ser Ala Phe Gln Arg Glu Leu Glu Arg Asp Ile Leu His Phe Leu
 290 295 300
 Thr Val Thr Met Arg Pro Ala Glu Ala Ala Ile Ser Gly
 305 310 315

<210> 39

<211> 496

<212> PRT

<213> Xanthomonas albilineans

<400> 39

Met Gln Lys Pro Lys Glu Ala Leu Gly Met Pro Pro Gly Met Ala Pro
 1 5 10 15
 Pro Gly Ala Gln Phe Asp Tyr Arg Trp Arg Trp Pro Ala Met Ile Val
 20 25 30
 Leu Leu Ser Ala Asn Phe Met Asn Leu Leu Asp Val Gly Ile Val Asn
 35 40 45

Val	Ala	Leu	Pro	Ser	Ile	Gln	Lys	Asn	Leu	Gly	Ala	Asp	Glu	Gln	Gln	50	55	60
Leu	Glu	Trp	Ile	Val	Ala	Ile	Tyr	Ile	Leu	Leu	Phe	Ala	Leu	Gly	Leu	65	70	75
Leu	Pro	Leu	Gly	Arg	Leu	Gly	Asp	Met	Leu	Gly	Arg	Lys	Arg	Met	Phe	85	90	95
Gly	Thr	Gly	Val	Ala	Gly	Phe	Ile	Leu	Met	Ser	Ala	Phe	Cys	Ala	Ile	100	105	110
Ala	Gly	Asn	Ile	His	Val	Leu	Ile	Ile	Ala	Arg	Ala	Leu	Gln	Gly	Leu	115	120	125
Ala	Ala	Ala	Met	Leu	Ala	Pro	Gln	Val	Met	Ala	Ile	Ala	Gln	Thr	Met	130	135	140
Phe	Ala	Pro	Lys	Glu	Arg	Ala	Ala	Ala	Phe	Ser	Leu	Phe	Gly	Leu	Val	145	150	155
Ala	Gly	Leu	Ala	Ser	Phe	Ala	Gly	Pro	Leu	Val	Ser	Gly	Leu	Leu	Ile	165	170	175
His	Ile	Asp	Ala	Phe	Gly	Val	Gly	Trp	Arg	Ala	Ile	Phe	Leu	Ile	Asn	180	185	190
Val	Pro	Ile	Gly	Leu	Val	Thr	Leu	Leu	Ala	Ala	Ala	Ile	Trp	Val	Pro	195	200	205
Lys	Val	Pro	Ala	His	Ala	Gly	Ile	His	Asn	Asp	Trp	Val	Gly	Ile	Ala	210	215	220
Leu	Ala	Ala	Leu	Ala	Leu	Leu	Cys	Leu	Val	Phe	Pro	Leu	Ile	Glu	Gly	225	230	235
Arg	Ala	Tyr	Gly	Trp	Pro	Leu	Trp	Cys	Phe	Ala	Ala	Ile	Ala	Leu	Gly	245	250	255
Ile	Pro	Leu	Leu	Val	Ala	Phe	Val	Ala	Trp	Gln	Arg	Arg	Gln	Ala	His	260	265	270
Leu	Ala	Arg	Pro	Ala	Leu	Leu	Pro	Ile	Tyr	Leu	Met	Ser	His	Arg	Asp	275	280	285
Tyr	Ile	Leu	Gly	Ala	Leu	Ser	Val	Ser	Val	Phe	Tyr	Ser	Ala	Leu	Gln	290	295	300
Gly	Phe	Phe	Leu	Val	Phe	Val	Ile	Phe	Leu	Gln	Gln	Gly	Leu	Ala	Tyr	305	310	315
Ser	Ala	Leu	Glu	Thr	Gly	Val	Ala	Thr	Thr	Pro	Phe	Pro	Val	Gly	Val	325	330	335
Ala	Ile	Ala	Ser	Met	Leu	Ala	Arg	His	Val	Glu	Ser	Leu	Arg	Ala	Lys	340	345	350
Ile	Phe	Ser	Gly	Ala	Cys	Leu	Met	Ile	Ala	Ser	Tyr	Leu	Ala	Leu	Trp	355	360	365

Val Ile Ile Thr Arg Ser Glu Gly Ser Leu Asp Pro Trp Thr Leu Thr
 370 375 380

Leu Pro Leu Leu Ile Gly Gly Leu Gly Cys Gly Ile Thr Ile Ala Ser
 385 390 395 400

Leu Phe Gln Thr Val Met Arg Thr Val Pro Leu Lys Asp Ala Gly Ala
 405 410 415

Gly Ser Gly Ala Leu Gln Val Ile Gln Gln Val Gly Gly Met Leu Gly
 420 425 430

Ile Ala Leu Val Ser Glu Ile Phe Phe Ser Gly Leu His Gln His Leu
 435 440 445

Gln Gly Pro Ala Gly Val Ala Leu Ala Phe Lys Gln Ala Phe Gly Ala
 450 455 460

Thr Val Val Tyr Tyr Ile Ala Ala Asn Ala Phe Val Ala Leu Ser Thr
 465 470 475 480

Leu Gly Leu Gln Phe Lys Leu Thr Gln Phe Ala Pro Gln Ser Ser Pro
 485 490 495

<210> 40
 <211> 584
 <212> PRT
 <213> Xanthomonas albilineans

<400> 40

Met Lys Arg Thr Tyr Ile Gly Leu Ala Asn Ser Phe His Asp Ser Ala
 1 5 10 15

Ile Ala Ile Val Gly Asp Asp Gly Gln Val Arg Phe Ala Glu Ala Thr
 20 25 30

Glu Arg Tyr Leu Gln Tyr Lys Arg Ser Ile Gly Val Ala Pro Asp Val
 35 40 45

Phe Gln Arg Ala Ile Lys Leu Val His Glu Tyr Gly Asp Pro Gly Ala
 50 55 60

Glu Leu Val Val Ala Thr Ser Trp Ser Gly Gln Thr Pro Glu Leu Met
 65 70 75 80

Arg Glu Gly Leu Gly Lys Thr Ala Gln Ala Val Asp Gln Tyr Arg Ser
 85 90 95

Ala Phe Gly Asp Leu Pro Trp His Val Asn Lys Gln Phe Val Ala Gln
 100 105 110

Ser Phe Phe Tyr Arg Ser Gln Leu Ala Met Val Glu His Pro Gly His
 115 120 125

Leu Leu Glu Tyr Asp Leu Ser His Met Ala Glu Pro Ala Phe Lys Pro
 130 135 140

Pro Ser Tyr Arg His Tyr Glu His His Leu Thr His Ala Val Ala Gly
 145 150 155 160

Cys	Tyr	Thr	Ser	Pro	Phe	Glu	Glu	Ala	Val	Cys	Ala	Val	Leu	Asp	Gly	
				165					170					175		
Met	Gly	Glu	Lys	Asn	Ala	Leu	Ala	Cys	Tyr	His	Tyr	Gln	Gln	Gly	Lys	
			180					185					190			
Leu	Thr	Pro	Ile	His	Gln	Ser	Glu	Thr	Ser	Ser	Trp	Ala	Ser	Leu	Gly	
		195					200					205				
Phe	Phe	Tyr	Gly	Met	Ile	Cys	Glu	Val	Cys	Gly	Phe	Gly	Thr	Leu	Ser	
	210					215					220					
Gly	Glu	Glu	Trp	Lys	Val	Met	Gly	Leu	Ala	Ala	Tyr	Gly	Gln	His	Asp	
225					230					235					240	
Arg	Gln	Leu	Tyr	Glu	Leu	Leu	Arg	Gln	Met	Leu	Arg	Val	Asp	Gly	Leu	
				245					250					255		
Thr	Leu	Arg	Phe	Ala	Pro	Ala	Ala	Gln	Phe	Ser	Gln	Leu	Gln	Arg	Thr	
			260					265					270			
Leu	Tyr	Ala	Met	Arg	Arg	Cys	Lys	Gly	Gln	Pro	Thr	Ile	Glu	Leu	Ala	
		275					280					285				
Asn	Leu	Ala	Tyr	Ala	Gly	Gln	Gln	Val	Phe	Cys	Asp	Val	Leu	Phe	Glu	
	290					295					300					
Phe	Leu	His	Asn	Leu	His	Ala	Leu	Gly	Leu	Ser	Asp	His	Leu	Val	Leu	
305					310					315					320	
Gly	Gly	Gly	Cys	Ala	Leu	Asn	Ser	Ser	Ala	Asn	Gly	Arg	Val	Leu	Ala	
				325					330					335		
Glu	Thr	Pro	Phe	Arg	His	Leu	His	Val	Phe	Ala	Ala	Pro	Gly	Asp	Asp	
			340					345					350			
Gly	Asn	Ala	Val	Gly	Ala	Ala	Leu	Trp	Ala	His	Ala	Glu	Asp	His	Pro	
		355					360					365				
Glu	Gln	Thr	Pro	Pro	Ala	Ala	Arg	Glu	Gln	Ser	Pro	Tyr	Leu	Gly	Ser	
	370					375					380					
Ser	Met	Ser	Ala	Glu	Thr	Leu	His	Asn	Val	Glu	Arg	Phe	Gly	Ala	Leu	
385					390					395					400	
Ser	Lys	Phe	Thr	Arg	Cys	Leu	Asp	Asp	Ala	Ala	Gln	Arg	Ala	Ala	Arg	
				405					410					415		
Leu	Leu	Thr	Glu	Gly	Lys	Ile	Val	Ala	Trp	Val	Gln	Gly	Arg	Ala	Glu	
			420					425					430			
Phe	Gly	Pro	Arg	Ala	Leu	Gly	Asn	Arg	Ser	Ile	Leu	Ala	Asp	Pro	Arg	
		435					440					445				
Ser	Pro	Ala	Ile	Lys	Asp	Ile	Ile	Asn	Ala	Arg	Val	Lys	Phe	Arg	Glu	
	450					455					460					
Glu	Phe	Arg	Pro	Phe	Ala	Pro	Ser	Ile	Leu	His	Glu	His	Gly	Ala	Glu	
465					470					475					480	

Tyr Phe Glu Leu Tyr Gln Glu Ser Pro Tyr Met Glu Arg Thr Leu Lys
 485 490 495
 Phe Arg Ala Glu Ala Thr Arg Lys Val Pro Gly Val Val His His Asp
 500 505 510
 Gly Thr Gly Arg Leu Gln Thr Val Lys Gln His Trp Asn Pro Arg Tyr
 515 520 525
 His Ala Leu Ile Lys Glu Phe Tyr Arg Leu Thr Gly Ile Pro Leu Val
 530 535 540
 Leu Asn Thr Ser Phe Asn Val Met Gly Lys Pro Ile Ala His Ser Val
 545 550 555 560
 Glu Asp Ala Leu Ser Ile Phe Phe Thr Ser Gly Leu Asp Ala Met Phe
 565 570 575
 Ile Asp Asp Val Leu Ile Glu Lys
 580

<210> 41
 <211> 88
 <212> PRT
 <213> Xanthomonas albilineans

<400> 41

Met Arg Thr Ser Lys Phe Asn Glu Thr Gln Ile Ile Ala Thr Leu Lys
 1 5 10 15
 Gln Ala Asp Ala Gly Val Pro Val Lys Asp Ile Cys Arg Gln Val Gly
 20 25 30
 Ile Ser Thr Ala Thr Tyr Tyr Gln Trp Lys Ser Lys Tyr Val Ala Ser
 35 40 45
 Glu Met Pro Ser Ser Arg His Thr Ser Leu Thr Trp Arg Pro Pro Ser
 50 55 60
 Thr Cys Phe Ser Val Ala Thr Ile Trp Leu Ser Val Asn Leu Leu Leu
 65 70 75 80
 Arg Ile Val Gly Arg Leu Gly Gly
 85

<210> 42
 <211> 716
 <212> PRT
 <213> Xanthomonas albilineans

<400> 42

Met Arg Cys Leu Ile Ile Asn Asn Tyr Asp Ser Phe Thr Trp Asn Leu
 1 5 10 15
 Ala Asp Tyr Val Ala Gln Ile Phe Gly Glu Asp Pro Leu Val Val His
 20 25 30

Asn Asp Glu Tyr Ser Trp His Glu Leu Lys Asp Arg Gly Gly Phe Ser
 35 40 45
 Ser Ile Ile Val Ser Pro Gly Pro Gly Ser Val Val Asn Glu Ala Asp
 50 55 60
 Phe His Ile Ser Leu Gln Ala Leu Glu Gln Asn Glu Phe Pro Val Leu
 65 70 75 80
 Gly Val Cys Leu Gly Phe Gln Gly Leu Ala His Val Tyr Gly Gly Arg
 85 90 95
 Ile Leu His Ala Pro Val Pro Phe His Gly Arg Arg Ser Thr Val Ile
 100 105 110
 Asn Thr Gly Asp Gly Leu Phe Glu Gly Ile Pro Gln Arg Phe Glu Ala
 115 120 125
 Val Arg Tyr His Ser Leu Met Val Cys Gln Gln Ser Leu Pro Pro Val
 130 135 140
 Leu Lys Val Thr Ala Arg Thr Asp Cys Gly Val Val Met Gly Leu Gln
 145 150 155 160
 His Val Gln His Pro Lys Trp Gly Val Gln Phe His Pro Glu Ser Ile
 165 170 175
 Leu Thr Glu His Gly Lys Arg Ile Val Ala Asn Phe Ala Lys Leu Ala
 180 185 190
 Ala Arg His Ser Ala Pro Leu Leu Ala Gly Ser Glu Gln Ala Gly Lys
 195 200 205
 Val Leu Ser Val Cys Ala Pro Glu Met Val Thr Pro Arg Val Arg Arg
 210 215 220
 Met Leu Ser Arg Lys Ile Lys Cys Arg Trp Gln Ala Glu Asp Val Phe
 225 230 235 240
 Leu Ala Leu Phe Ala Asp Glu Lys His Cys Phe Trp Leu Asp Ser Gln
 245 250 255
 Leu Val Cys Ser Pro Met Ala Arg Tyr Ser Phe Met Gly Ala Val Asn
 260 265 270
 Glu Ser Glu Val Val Arg His Cys Val Arg Pro Gly Ser Met Val Gln
 275 280 285
 Glu Ala Gly Glu Arg Phe Leu Ala Glu Met Asp Arg Ala Leu Gln Ser
 290 295 300
 Val Leu Thr Glu Asp Val Ala Glu Arg Pro Pro Phe Ala Phe Arg Gly
 305 310 315 320
 Gly Tyr Val Gly Tyr Met Ser Tyr Glu Met Lys Ser Val Phe Gly Ala
 325 330 335
 Pro Ala Ser His Ala Asn Ala Ile Pro Asp Ala Leu Trp Met Arg Val
 340 345 350
 Glu Arg Phe Val Ala Phe Asp His Ala Thr Glu Glu Val Trp Leu Leu

355					360					365					
Ala	Leu	Ala	Asp	Thr	Glu	Asp	Leu	Ser	Ala	Leu	Ala	Trp	Leu	Asp	Ala
370					375					380					
Ile	Glu	Gln	Arg	Ile	His	Ala	Ile	Gly	Gln	Ala	Ala	Pro	Ala	Cys	Ile
385					390					395					400
Ser	Leu	Gly	Leu	Arg	Ser	Met	Glu	Ile	Glu	Leu	Asn	His	Gly	Arg	Arg
				405					410					415	
Gly	Tyr	Leu	Glu	Ala	Ile	Glu	Arg	Cys	Lys	Gln	Arg	Ile	Val	Asp	Gly
			420					425					430		
Glu	Ser	Tyr	Glu	Ile	Cys	Leu	Thr	Asp	Leu	Phe	Ser	Phe	Gln	Ala	Glu
		435					440					445			
Leu	Asp	Pro	Leu	Met	Leu	Tyr	Arg	Tyr	Met	Arg	Arg	Gly	Asn	Pro	Ala
	450					455					460				
Pro	Phe	Gly	Ala	Tyr	Leu	Arg	Asn	Gly	Ser	Asp	Cys	Ile	Leu	Ser	Thr
465					470					475					480
Ser	Pro	Glu	Arg	Phe	Leu	Glu	Val	Asp	Gly	His	Gly	Thr	Ile	Gln	Thr
				485					490					495	
Lys	Pro	Ile	Lys	Gly	Thr	Cys	Arg	Arg	Ala	Glu	Asp	Pro	Gln	Leu	Asp
			500					505					510		
Arg	Asn	Leu	Ala	Met	Arg	Leu	Ala	Ala	Ser	Glu	Lys	Asp	Arg	Ala	Glu
		515					520					525			
Asn	Leu	Met	Ile	Val	Asp	Leu	Met	Arg	Asn	Asp	Leu	Ser	Arg	Val	Ala
	530					535					540				
Val	Pro	Gly	Ser	Val	Thr	Val	Pro	Lys	Leu	Met	Asp	Ile	Glu	Ser	Tyr
545					550					555					560
Lys	Thr	Val	His	Gln	Met	Val	Ser	Thr	Val	Glu	Ala	Arg	Leu	Arg	Ala
				565					570					575	
Asp	Cys	Ser	Leu	Val	Asp	Leu	Leu	Lys	Ala	Val	Phe	Pro	Gly	Gly	Ser
			580					585					590		
Ile	Thr	Gly	Ala	Pro	Lys	Leu	Arg	Ser	Met	Glu	Ile	Ile	Asp	Gly	Leu
		595					600					605			
Glu	Asn	Ala	Pro	Arg	Gly	Val	Tyr	Cys	Gly	Ser	Ile	Gly	Tyr	Leu	Gly
	610					615					620				
Tyr	Asn	Cys	Val	Ala	Asp	Leu	Asn	Ile	Ala	Ile	Arg	Ser	Leu	Ser	Tyr
625					630					635					640
Asp	Gly	Gln	Glu	Ile	Arg	Phe	Gly	Ala	Gly	Gly	Ala	Ile	Thr	Phe	Leu
				645					650					655	
Ser	Asp	Pro	Gln	Asp	Glu	Phe	Asp	Glu	Val	Leu	Leu	Lys	Ala	Glu	Ala
			660					665					670		
Ile	Leu	Lys	Pro	Ile	Trp	His	Tyr	Leu	His	Ala	Pro	Asn	Thr	Pro	Leu
		675					680					685			

His Tyr Glu Leu Arg Glu Asp Lys Leu Leu Leu Ala Glu His Cys Val
 690 695 700

Ser Glu Met Pro Ala Arg Gln Ala Phe Ile Glu Pro
 705 710 715

<210> 43
 <211> 137
 <212> PRT
 <213> Xanthomonas albilineans
 <400> 43

Met Arg Pro Pro Arg Leu Arg Ala Asn Gln Asp Gly Leu Leu Met Asp
 1 5 10 15

Thr Ala Gly Arg Val Val Glu Gly Cys Thr Ser Asn Leu Phe Leu Val
 20 25 30

Glu Asn Gly His Leu Val Thr Pro Asp Leu Gly Val Ala Gly Val Ser
 35 40 45

Gly Ile Met Arg Gly Arg Val Ile Glu Tyr Gly Arg Gln His Gly Leu
 50 55 60
 Ala Cys Ala Val Lys His Val Tyr Pro Asp Gln Leu Val Arg Ala Gln
 65 70 75 80

Glu Val Phe Leu Thr Asn Ala Val Phe Gly Ile Leu Leu Val Arg Ser
 85 90 95

Ile Asp Ala His Ser Tyr Arg Ile Asp Pro Val Thr Leu Arg Leu Leu
 100 105 110

Asp Ala Leu Cys Gln Gly Val Tyr Phe Thr Glu Arg Ser Leu His Gln
 115 120 125

Val Ser Thr His Ala Gly Gln Asp Pro
 130 135

<210> 44
 <211> 200
 <212> PRT
 <213> Xanthomonas albilineans
 <400> 44

Met Pro Ala Lys Thr Leu Glu Ser Lys Asp Tyr Cys Gly Glu Ser Phe
 1 5 10 15

Val Ser Glu Asp Arg Ser Gly Gln Ser Leu Glu Ser Ile Arg Phe Glu
 20 25 30

Asp Cys Thr Phe Arg Gln Cys Asn Phe Thr Glu Ala Glu Leu Asn Arg
 35 40 45

Cys Lys Phe Arg Glu Cys Glu Phe Val Asp Cys Asn Leu Ser Leu Ile
 50 55 60

Ser Ile Pro Gln Thr Ser Phe Met Glu Val Arg Phe Val Asp Cys Lys

65		70		75		80									
Met	Leu	Gly	Val	Asn	Trp	Thr	Ser	Ala	Gln	Trp	Pro	Ser	Val	Lys	Met
				85					90					95	
Glu	Gly	Ala	Leu	Ser	Phe	Glu	Arg	Cys	Ile	Leu	Asn	Asp	Ser	Leu	Phe
			100					105					110		
Tyr	Gly	Leu	Tyr	Leu	Ala	Gly	Val	Lys	Met	Val	Glu	Cys	Arg	Ile	His
		115					120					125			
Asp	Ala	Asn	Phe	Thr	Glu	Ala	Asp	Cys	Glu	Asp	Ala	Asp	Phe	Thr	Gln
	130					135					140				
Ser	Asp	Leu	Lys	Gly	Ser	Thr	Phe	His	Asn	Thr	Lys	Leu	Thr	Gly	Ala
145					150					155					160
Ser	Phe	Ile	Asp	Ala	Val	Asn	Tyr	His	Ile	Asp	Ile	Phe	His	Asn	Asp
			165						170					175	
Ile	Lys	Arg	Ala	Arg	Phe	Ser	Leu	Pro	Glu	Ala	Ala	Ser	Leu	Leu	Asn
			180					185						190	
Ser	Leu	Asp	Ile	Glu	Leu	Ser	Asp								
		195					200								

<210> 45
 <211> 202
 <212> PRT
 <213> Xanthomonas albilineans

<400> 45

Met	His	Pro	Pro	Ser	Pro	Leu	Asn	Thr	Gln	Gln	Lys	Asp	Trp	Leu	Thr
1				5					10					15	
Arg	Gly	Gly	Ser	Leu	Thr	Ala	His	Leu	Arg	Leu	Leu	Gly	Gln	Val	Gln
			20					25					30		
Val	Gln	Val	Gln	Arg	Glu	His	Lys	Ser	Met	Ala	Trp	Leu	Asp	Glu	Tyr
		35					40					45			
Arg	Val	Leu	Gly	Leu	Ser	Arg	Cys	Leu	Leu	Val	Trp	Val	Arg	Glu	Val
	50					55					60				
Val	Leu	Val	Val	Asp	Ala	Lys	Pro	Tyr	Val	Tyr	Ala	Arg	Ser	Leu	Thr
65					70					75					80
Pro	Leu	Thr	Ala	Ser	Tyr	Asn	Ala	Trp	Gln	Ala	Val	Arg	Ser	Ile	Gly
				85					90					95	
Ser	Arg	Pro	Leu	Ala	Asp	Leu	Leu	Phe	Arg	Asp	Arg	Ser	Val	Leu	Arg
			100					105					110		
Ser	Ala	Leu	Ala	Ser	Arg	Arg	Ile	Thr	Ala	Gln	His	Pro	Leu	His	Arg
		115					120					125			
Arg	Ala	Cys	Asn	Phe	Val	Ala	Gln	Ser	His	Ala	Thr	Gln	Ala	Leu	Leu
	130					135					140				

Ala Arg Arg Ser Val Phe Thr Arg Gln Gly Ala Pro Leu Leu Ile Thr
 145 150 155 160

Glu Cys Met Leu Pro Ala Leu Trp Ala Thr Leu Glu Pro Val Ala Ala
 165 170 175

Pro Arg Gln Ala Ser Leu Ser Ala Asp Gly Pro Cys Arg His Ser Ala
 180 185 190

Gln Ile Val Ser Pro Glu Ser Met Leu Glu
 195 200

<210> 46
 <211> 278
 <212> PRT
 <213> Xanthomonas albilineans

<400> 46

Met Pro Asn Ala Val Pro Met Gln Gly Ala Arg Gly Leu Pro Gln Pro
 1 5 10 15

Gln Ala Met Asn Pro Gly Leu Pro Ser Val Gly Gly Leu Ser Ala Gly
 20 25 30

Gln Pro Leu Gln Leu Ser Leu Ala Pro Glu Leu Gln Ala Ala Ala Arg
 35 40 45

Ser Ala His Arg His Leu Leu Asp Asp Gly Thr Ala Leu Tyr Leu Leu
 50 55 60

Ala Phe Asp Thr Ala Gln Phe Asp Pro Gly Ala Phe Ala Ala Met Ala
 65 70 75 80

Ile Ala Arg Pro Asp Ser Ile Ala Arg Ser Val Arg Lys Arg Gln Ala
 85 90 95

Glu Phe Leu Phe Gly Arg Leu Ala Ala Arg Leu Ala Leu Gln Glu Val
 100 105 110

Leu Gly Pro Ala Gln Ala Gln Ala Asp Ile Ala Ile Gly Ala Thr Arg
 115 120 125

Ala Pro Cys Trp Pro Ala Gly Ser Leu Gly Ser Ile Ser His Cys Glu
 130 135 140

Asp Tyr Ala Ala Ala Ile Ala Met Ala Ala Gly Thr Arg His Gly Val
 145 150 155 160

Gly Ile Asp Leu Glu Arg Pro Ile Thr Pro Ala Ala Arg Ala Ala Leu
 165 170 175

Leu Ser Ile Ala Ile Asp Ala Asp Glu Ala Ala Arg Leu Ala Lys Ala
 180 185 190

Ala Asp Ala Gln Trp Pro Gln Asp Leu Leu Leu Thr Ala Leu Phe Ser
 195 200 205

Ala Lys Glu Ser Leu Phe Lys Ala Ala Tyr Ser Ala Val Gly Arg Tyr
 210 215 220

Phe Asp Phe Ser Ala Ala Arg Leu Cys Gly Ile Asp Leu Ala Arg Gln
 225 230 235 240
 Cys Leu His Leu Arg Leu Thr Glu Thr Leu Cys Ala Gln Phe Val Ala
 245 250 255
 Gly Gln Val Cys Glu Val Gly Phe Ala Arg Leu Pro Pro Asp Leu Val
 260 265 270
 Leu Thr His Tyr Ala Trp
 275

<210> 47
 <211> 634
 <212> PRT
 <213> Xanthomonas albilineans

<400> 47

Met Ser Val Glu Thr Gln Lys Glu Thr Leu Gly Phe Gln Thr Glu Val
 1 5 10 15
 Lys Gln Leu Leu Gln Leu Met Ile His Ser Leu Tyr Ser Asn Lys Glu
 20 25 30
 Ile Phe Leu Arg Glu Leu Ile Ser Asn Ala Ser Asp Ala Ala Asp Lys
 35 40 45
 Leu Arg Phe Glu Ala Leu Val Lys Pro Glu Leu Leu Asp Gly Asp Ala
 50 55 60
 Gln Leu Arg Ile Arg Ile Gly Phe Asp Lys Asp Ala Gly Thr Val Thr
 65 70 75 80
 Ile Asp Asp Asn Gly Ile Gly Met Ser Arg Glu Glu Ile Val Ala His
 85 90 95
 Leu Gly Thr Ile Ala Lys Ser Gly Thr Ser Asp Phe Leu Lys His Leu
 100 105 110
 Ser Gly Asp Gln Lys Lys Asp Ser His Leu Ile Gly Gln Phe Gly Val
 115 120 125
 Gly Phe Tyr Ser Ala Phe Ile Val Ala Asp Gln Val Asp Val Tyr Ser
 130 135 140
 Arg Arg Ala Gly Leu Pro Ala Ser Asp Gly Val His Trp Ser Ser Arg
 145 150 155 160
 Gly Glu Gly Glu Phe Glu Val Ala Thr Ile Asp Lys Pro Glu Arg Gly
 165 170 175
 Thr Arg Ile Val Leu His Leu Lys Glu Glu Glu Lys Gly Phe Ala Asp
 180 185 190
 Gly Trp Lys Leu Arg Ser Ile Val Arg Lys Tyr Ser Asp His Ile Ala
 195 200 205
 Leu Pro Ile Glu Leu Ile Lys Glu His Tyr Gly Glu Asp Lys Asp Lys

210	215	220
Pro Glu Thr Pro Glu Trp Glu Thr Val Asn Arg Ala Ser Ala Leu Trp 225 230 235 240		
Thr Arg Pro Arg Thr Glu Ile Lys Asp Glu Glu Tyr Gln Glu Leu Tyr 245 250 255		
Lys His Ile Ala His Asp His Glu Asn Pro Val Ala Trp Ser His Asn 260 265 270		
Lys Val Glu Gly Lys Leu Glu Tyr Thr Ser Leu Leu Tyr Leu Pro Gly 275 280 285		
Arg Ala Pro Phe Asp Leu Tyr Gln Arg Asp Ala Ser Arg Gly Leu Lys 290 295 300		
Leu Tyr Val Gln Arg Val Phe Ile Met Asp Gln Ala Asp Gln Phe Leu 305 310 315 320		
Pro Leu Tyr Leu Arg Phe Ile Lys Gly Ile Val Asp Ser Ser Asp Leu 325 330 335		
Pro Leu Asn Val Ser Arg Glu Ile Leu Gln Ser Gly Pro Val Ile Asp 340 345 350		
Ser Met Lys Ser Ala Leu Thr Lys Arg Ala Leu Asp Met Leu Glu Lys 355 360 365		
Leu Ala Lys Asp Asp Pro Glu Arg Tyr Lys Gly Val Trp Lys Asn Phe 370 375 380		
Gly Gln Val Leu Lys Glu Gly Pro Ala Gln Asp Phe Gly Asn Arg Glu 385 390 395 400		
Lys Ile Ala Gly Leu Leu Arg Phe Ala Ser Thr His Ser Gly Asp Asp 405 410 415		
Ala Gln Asn Val Ser Leu Ala Asp Tyr Val Ala Arg Met Lys Asp Gly 420 425 430		
Gln Asp Lys Leu Tyr Tyr Leu Thr Gly Glu Ser Tyr Ala Gln Ile Lys 435 440 445		
Asp Ser Pro His Leu Glu Val Phe Arg Lys Lys Gly Ile Glu Val Leu 450 455 460		
Leu Leu Thr Asp Arg Ile Asp Glu Trp Leu Met Ser Tyr Leu Thr Glu 465 470 475 480		
Phe Asp Ser Lys Ser Phe Val Asp Val Ala Arg Gly Asp Leu Asp Leu 485 490 495		
Gly Lys Leu Asp Ser Glu Glu Glu Lys Gln Ala Gln Glu Glu Ala Ala 500 505 510		
Lys Ala Lys Gln Gly Leu Ala Glu Arg Ile Gln Gln Val Leu Lys Asp 515 520 525		
Glu Val Ala Glu Val Arg Val Ser His Arg Leu Thr Asp Ser Pro Ala 530 535 540		

Ile Leu Ala Ile Gly Gln Gly Asp Met Gly Leu Gln Met Arg Gln Ile
545 550 555 560

Leu Glu Ala Ser Gly Gln Lys Leu Pro Glu Ser Lys Pro Val Phe Glu
565 570 575

Phe Asn Pro Ala His Pro Leu Ile Glu Lys Leu Asp Ala Glu Pro Asp
580 585 590

Val Asp Arg Phe Gly Asp Leu Ala Arg Val Leu Phe Asp Gln Ala Ala
595 600 605

Leu Ala Ala Gly Asp Ser Leu Lys Asp Pro Ala Ala Tyr Val Arg Arg
610 615 620

Leu Asn Lys Leu Leu Leu Glu Leu Ser Ala
625 630

<210> 48
<211> 20
<212> DNA
<213> Xanthomonas albilineans

<400> 48

gcgtaccggt gtccagtagg 20

<210> 49
<211> 20
<212> DNA
<213> Xanthomonas albilineans

<400> 49

gctggaaacc gagaatctga 20

<210> 50
<211> 20
<212> DNA
<213> Xanthomonas albilineans

<400> 50

gacacgatca gccgctagga 20

<210> 51
<211> 20
<212> DNA
<213> Xanthomonas albilineans

<400> 51

accagcagtt gggccagcct 20

<210> 52

<211> 19
<212> DNA
<213> Xanthomonas albilineans

<400> 52

tgccacacagg ccgtcgagt 19

<210> 53
<211> 20
<212> DNA
<213> Xanthomonas albilineans

<400> 53

gcgagaggac aagctgctgc 20

<210> 54
<211> 20
<212> DNA
<213> Xanthomonas albilineans

<400> 54

cgttgaggat gcagcgctcg 20